IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES WHEN THE DISTRIBUTION OF EFFECT SIZES IS ASYMMETRIC IN TWO CLASS EXPERIMENTS

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

High-throughput RNA Sequencing (RNA-Seq) has emerged as an innovative and powerful technology for detecting differentially expressed genes (DE) across different conditions. Unlike continuous microarray data, RNA-Seq data consist of discrete read counts mapped to a particular gene. Most proposed methods for detecting DE genes from RNA-Seq are based on statistics that compare normalized read counts between conditions. However, most of these methods do not take into account potential asymmetry in the distribution of effect sizes. In this dissertation, we propose methods to detect DE genes when the distribution of the effect sizes is observed to be asymmetric. These proposed methods improve detection of differential expression compared to existing methods. Chapter 3 proposes two new methods that modify an existing nonparametric method, Significance Analysis of Microarrays with emphasis on RNA-Seq data (SAMseq), to account for the asymmetry in the distribution of the effect sizes. Results of the simulation studies indicates that the proposed methods, compared to the SAMseq method identifies more DE genes, while adequately controlling false discovery rate (FDR). Furthermore, the use of the proposed methods is illustrated by analyzing a real RNA-Seq data set containing two different mouse strain samples. In Chapter 4, additional simulation studies are performed to show that the one of the proposed method, compared with other existing methods, provides better power for identifying truly DE genes or more sufficiently controls FDR in most settings where asymmetry is present. Chapter 5 compares the performance of parametric methods, DESeq2, NBPSeq and edgeR when there exist asymmetric effect sizes and the analysis takes into account this asymmetry. Through simulation studies, the performance of these methods are compared to the traditional BH and q-value method in the identification of DE genes. This research proposes a new method that modifies these parametric methods to account for

asymmetry found in the distribution of effect sizes. Likewise, illustration on the use of these parametric methods and the proposed method by analyzing a real RNA-Seq data set containing two different mouse strain samples. Lastly, overall conclusions are given in Chapter 6.

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DEDICATION

I dedicated this dissertation to my late grandparents.

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LIST OF ABBREVIATIONS

DE	Differentially expressed.
EE	Equally expressed.
FWER	Familywise error rate.
FDR	False discovery ratio.
BH	Benjamini and Hochberg.
pFDR	Positive false discovery.
DDE	Declared differentially expressed.
SAMseq	Significance analysis of microarrays using sequencing data
DRG	Dorsal root ganglion
SNL	Spinal nerve ligation

CHAPTER 1. INTRODUCTION

1.1. Background

Recent advances in technology have allowed the state of diseases and biological conditions to be characterized by distinct patterns of gene expression (Brown and Botstein, 1999; DeRisi et al., 1997; Eisen and Brown, 1999; Spellman et al., 1998). The development of DNA microarrays in the 1990s, has been the main technology for large-scale studies in measuring gene expression (i.e., quantifying the amount of messenger RNA transcripts for a gene) in experimental units (referred to as “experiments”) in the field of genetic, biological and medical research (Macgregor and Squire, 2002; Petricoin et al., 2002). This technology has the ability to simultaneously measure tens of thousands of transcripts to provide information in dealing with a wide range of biological problems, including the identification of genes that are differentially expressed between diseased and healthy tissues, new insights into developmental processes, and the evolution of gene regulation in different species (Baldi and Hatfield, 2002; Kerr et al., 2008; Passador-Gurgel et al., 2007). Although microarrays are still the most common and affordable technology used in transcript profiling, it has several limitations. For example, background hybridization limits the accuracy of gene expression measurements, particularly for transcripts present in low abundance. Also, probes differ significantly in their hybridization properties, and arrays are limited to measuring only genes for which probes are designed (Abdullah-Sayani et al., 2006; Russo et al., 2003).

In recent years, a new approach known as RNA Sequencing (RNA-Seq), that is, the direct sequencing of transcripts by high-throughput sequencing technologies, has been developed (Nagalakshmi et al., 2008; Wilhelm and Landry, 2009) to measure the entire transcriptome. It has been shown to have the potential to become a replacement to microarrays for whole-genome transcriptome profiling (Beyer et al., 2012; Montgomery et al., 2010; A. Mortazavi et al., 2008; Mutz et al., 2013; Nagalakshmi et al., 2001). RNA-Seq uses the capabilities of next-generation sequencing to reveal the presence and quantity of RNA expressions from a genome and is more preferable compared to microarray approaches because it provides more information such as alternative splicing and isoform-specific gene expression with low background signal (Chu and Corey, 2012; Wang et al., 2009). These sequencing methods also offer more accurate quantification of expression levels compared to other technologies. The development of sequencing technologies enables simultaneous sequencing of millions of molecules; leading to advanced approaches for measuring expression levels (Bennett et al., 2005; Margulies et al., 2005) with high accuracy and reproducibility (Fu et al., 2009; Marioni et al., 2008b; Miller et al., 2008; Ali Mortazavi et al., 2008). Researchers often use RNA-Seq to identify differentially expressed genes (DE) genes in many types of comparative studies. Also, RNA-Seq does not depend on genome annotation for prior probe selection and avoids the biases introduced during hybridization of microarrays. However, RNA-Seq poses algorithmic and logistical challenges for data analysis and storage. Although many computational methods have been developed for alignment of reads, quantification of genes and transcripts, and identification of differentially expressed genes (Garber et al., 2011), there is great variability in the development of these available computational tools. Further details on RNA-Seq technology and its challenges, benefits and applications are reviewed elsewhere (Bloom et al., 2009; Bradford et al., 2010; Hurd and Nelson, 2009; Malone and Oliver, 2011; Wang et al., 2009).

1.2. Research objectives

This research is specific to analyzing gene expression data sets with two class experiments. An example includes an experiment comparing healthy patients to those with an illness or disease. The goals of this research are to:

(1) Develop methods for analyzing RNA-Seq data that takes into account asymmetry in the distribution of the test statistic when analyzing RNA expression data that lead to an improvement over previously existing methods in the number of truly DE genes identified as differentially expressed, while still adequately controlling false discovery rate. A simulation study will be performed to determine under which experimental settings taking into account asymmetry in the distribution of the test statistics improves identification of DE genes compared to traditional methods and by reanalyzing data generated by real RNA-Seq experiments.

(2) Compare the best-performing proposed method to other commonly-used existing methods for identifying DE genes from RNA-Seq experiments. These methods are NBPSeq (Yanming et al., 2011), edgeR (Robinson et al., 2010), and DESeq2 (Love et al., 2014). Similar to goal (1), comparison of methods are accomplished through simulation studies and the use of these methods are illustrated by reanalyzing data generated from real RNA-Seq experiments.

(3) Lastly, this research compares the performance of these commonly-used existing methods for identifying DE genes from RNA-Seq experiments when there exists asymmetry in the distribution of effect sizes, using BH method proposed by Benjamini and Hochberg (1995) and q-value method proposed by Storey (2002) to adequately control false discovery rate. Similar to goals (1) and (2), comparison

of these methods is accomplished through simulation studies and illustrated by reanalyzing data generated from real RNA-Seq experiments

1.3. Organization

The rest of the dissertation is organized as follows. In Chapter 2, RNA-Sequencing analysis and multiple hypothesis testing with emphasis on false discovery rate are reviewed. Chapter 3 describes the SAMseq method for two class experiments and two proposed methods that modify this procedure in estimating FDR are presented. A description and the results of simulation studies implemented to compare the performances of the proposed methods and traditional SAMseq method, in terms of identification of differential expressed genes and FDR control, are presented. Analysis of a real RNA-Seq experiment using all methods from the simulation studies, conclusions and recommendations are discussed. Chapter 4 describes and presents the results of simulation studies implemented to compare the performances of the best-performing proposed method and the three existing methods in terms of identification of differential expressed genes and FDR control. Chapter 5 briefly describes the DESeq2, NBPSeq, edgeR methods and presents methods that modify the procedures used in adjusting the p-value when estimating FDR. A description and the results of simulation studies implemented to evaluate the performances of the proposed method and these parametric methods, in terms of identification of differential expressed genes and FDR control are presented. Analysis of a real RNA-Seq dataset using all methods from the simulation studies, conclusions and recommendations are discussed. All analyses are performed in R. Lastly, overall conclusions of this research are given Chapter 6.

CHAPTER 2. LITERATURE REVIEW

2.1. Performance of RNA – sequencing analysis

Several studies comparing RNA-Seq and hybridization-based arrays have been performed (Fu et al., 2009; Marioni et al., 2008a; Sirbu et al., 2012). Marioni et al. (2008a) and estimated the technical variance associated with Illumina RNA-Sequencing to identify DE genes with existing array technologies. The results indicated that, RNA-Seq data on the Illumina platform was highly reproducible, with relatively low technical variation. The DE genes identified from RNA-Seq experiments were similar to those identified using microarrays. Fu et al. (2009) designed a study that used protein expression measurements to evaluate the accuracy of microarrays and RNA-Seq for mRNA quantification. In that study, gene expression levels were measured using Shotgun Mass Spectroscopy. This allowed for assessment of the relative accuracy of the two transcriptome quantification approaches with respect to absolute transcript level measurements. The results from this study showed that RNA-Seq provided better estimates of the absolute transcript levels. Many recent studies have been performed to run RNA-Seq and microarray in parallel with a focus on finding the relationship between them (Bottomly et al., 2011; Sirbu et al., 2012; Zhang et al., 2012).

2.2. Multiple testing

A major challenge faced by researchers in the analysis of large data sets is the problem of multiple testing. In RNA-sequencing analysis and other gene expression analysis, it is not unusual to test thousands of hypotheses simultaneously. For every hypothesis test, there is a risk of falsely rejecting a null hypothesis that is true, that is a Type I error, and of failing to reject a null hypothesis that is false, that is a Type II error. Traditionally, Type I errors are considered more problematic than Type II errors. The key goal of multiple testing methods is to control the rate at which Type I errors occur when many hypothesis tests are performed simultaneously.

The Family-Wise Error Rate (FWER) is often the preferred error rate to be controlled. Common procedures for identifying DE genes while controlling the FWER are the Bonferroni (Simes, 1986) and Holm (Holm, 1979) methods. However, for high-dimensional data in which thousands of hypotheses are being tested simultaneously, the FWER generally results in extremely low statistical power for identifying DE genes. In efforts to improve the power of detecting DE genes while still controlling multiple testing error, the False Discovery Rate (FDR) was developed (Benjamini and Hochberg, 1995).

2.3. False discovery rate

Many methods have been developed to overcome the problems that arise from multiple testing, and they all attempt to assign an adjusted p-value to each hypothesis test, or reduce the p-value threshold. Several traditional methods such as the Bonferroni correction are too conservative, as it reduces the number of false positives but also considerably decreases the number of true discoveries in many cases. FDR methods also determine adjusted p-values for each hypothesis test. More specifically, the FDR controls the proportion of false discoveries among all tests that are significant and has a greater power to determine truly significant results. This approach was proposed by Benjamini and Hochberg (1995) as a multiple-hypothesis testing error measure to control the proportion of Type I errors among all rejected null hypotheses (Benjamini and Hochberg, 1995). Benjamin and Hochberg (BH) considered the case of testing m null hypothesis, of which are true. Table 1 provides notation for random variables associated with different scenarios in a multiple testing experiment.

Table 1. Random Variables Corresponding to the Number of Errors Committed when Testing *m* Hypothesis

	Declared non-significant	Declared Significant	Total
True null hypothesis	U	V	m_0
Non - true null hypothesis	T	S	$m-m_0$
Total	$m-R$	R	m

BH defined the FDR as

$$FDR = E\left(\frac{V}{\max(R,1)}\right),. \tag{2.1}$$

and the following sequential p-value methods was provided to control the FDR. Let $p_1 \le p_2 \le ... \le p_m$ be the ordered p-values and let H_i be the null hypothesis of the i^{th} gene with corresponding p-value p_i. Also, let *k* be the largest *i* for which

$$p_i \le \frac{i}{m} q^* . \tag{2.2}$$

If all H_i, for $i = 1,2,...,k$ are rejected, then the above formula controls the FDR at q^* for any genes with true null hypotheses and any configuration of false null hypotheses. Also, if the test statistics corresponding to true null hypotheses are statistically independent, equation (2.2) controls FDR when

$$FDR \le \left(\frac{m_0}{m}\right) q^* \le q^* . \tag{2.3}$$

Figure 1 below shows the comparison between the controlling procedures used in FDR and FWER.

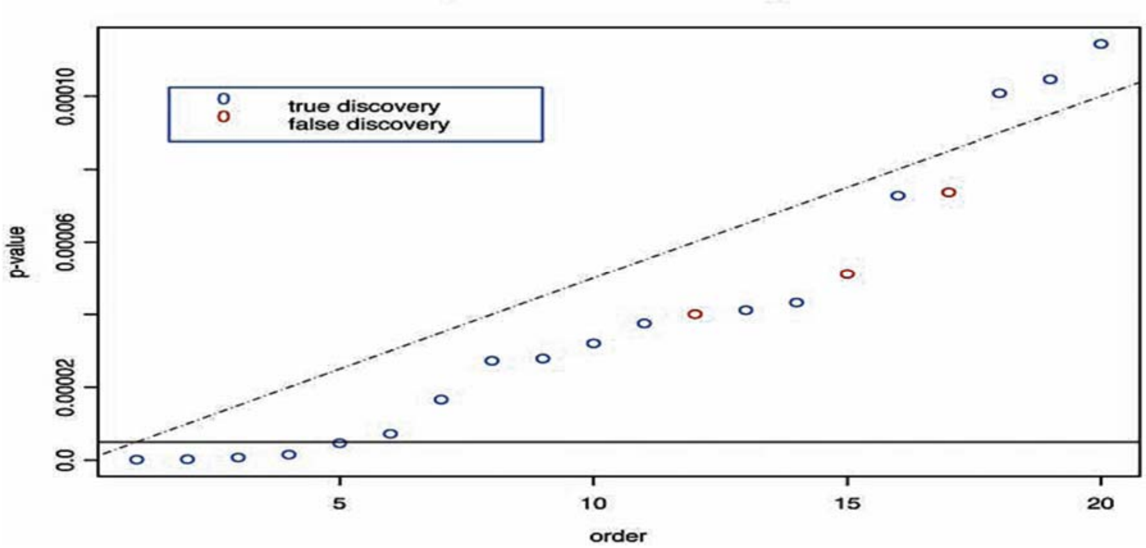

Figure 1. Comparison of the controlling procedures of FDR and FWER (Lazar, 2012)

Figure 1 above is a plot of the first 20 ordered p-values for a gene expression experiment, with the order indicator on the x-axis and p-values on the y-axis (Lazar, 2012). The horizontal solid line represents the Bonferroni correction method (controlling procedure for FWER) and the dashed line represents the FDR-controlling procedure. Points that fall below the line for a given method are considered to be significant by that method. From this plot, it is clear that the FDR controlling procedures allows for more tests to be identified as significant compared to the Bonferroni correction method. Thus, FDR-controlling methods result in higher power for detecting DE genes but also allow for more type I errors or false discoveries than the FWER. Storey (2002) pointed out the weaknesses in controlling the FDR which was proposed by BH and suggested that the FDR should be calculated as

$$pFDR = E\left(\frac{V}{R}\middle| R > 0\right), \tag{2.4}$$

where *p*FDR is the positive false discovery rate (Storey, 2002).

2.4. Q - value

Storey (2002), later developed the q-value, a natural *p*FDR analogue of the p-value, as a hypothesis testing error measure for each of the observed statistics with respect to *p*FDR (Storey, 2002). The q-value is the j^{th} smallest p-value p_j and is defined as

$$q_{(j)} = \min\left\{ \frac{p_{(r)}\hat{m}_0}{r} : r = j,\ldots,m \right\}, \tag{2.5}$$

where $p_{(r)}\hat{m}_0$ is an estimate of the number of false discoveries and r is the total number of genes declared to be DE if all genes with p-values less than or equal to p_r are declared DE. $\hat{m}_0$ is the estimate of the number of EE genes in a data set, and calculated using a method proposed by (Storey *et.al.*, 2003). This procedure involves first ordering all the p-values and estimating $\hat{m}_0(\lambda)$ for a range of λ between 0 and 1, where

$$\hat{m}_0(\lambda) = \frac{\sum_{j=1}^{m}\left\{p_j > \lambda\right\}}{(1-\lambda)}. \tag{2.6}$$

Then, a natural cubic spline is fit to the points $(\lambda, \hat{m}_0(\lambda))$. Finally, this function is evaluated at $\lambda = 1$ to obtain the final estimate of m_0 (Storey *et.al.*, 2003).

2.5. Asymmetric Q - value

Recently, Orr et al. (2014) suggested that when asymmetry in the distribution of test statistics is observed in a two class gene expression experiments, the estimation of FDR using the q-value method might be improved if this asymmetry is taken into consideration. The following method for doing this was proposed. Consider performing *m* hypothesis tests in the two class experiments (t = 1, 2). The null hypothesis for the j^{th} gene is $H_j : \mu_{1j} = \mu_{2j}$, where μ_{tj} is the population mean expression for gene j (j = 1, …, m) in experiment t. For each gene, an appropriate t-test statistic t_j is computed with its corresponding two-sided p-value obtained. The number of EE genes is then estimated as $\hat{m}_0$ using all m p-values using the methods described in Storey and Tibshirani (2003). Next, the p-values are then partitioned into two subsets based on the signs of the corresponding test statistics, $\left\{ p_k^{(1)} : k = 1,\ldots,m^- \right\}$ and $\left\{ p_k^{(2)} : k = 1,\ldots,m^+ \right\}$. This represent the subsets of ordered p-values corresponding to the m^- genes with negative statistics and the m^+ genes positive test statistics, respectively (Orr et al., 2014). Then, the q-values for each subset are estimated separately as

$$q_{(k)}^{(1)} = \min \left\{ \frac{p_{(r)}^{(1)} \hat{m}_0 / 2}{r} : r = k,\ldots,m^- \right\} \tag{2.7}$$

and

$$q_{(k)}^{(2)} = \min \left\{ \frac{p_{(r)}^{(1)} \hat{m}_0 / 2}{r} : r = k,\ldots,m^+ \right\}. \tag{2.8}$$

Simulation studies showed that this method improved the identification of DE genes over the traditional q-value method while adequately controlling FDR in when asymmetry was present in the distribution of the test statistics. Orr et al. (2014) also recommended the use of the proposed method in analyzing experiments with smaller sample sizes $(n \leq 10)$.

CHAPTER 3. MODIFYING SAMseq TO ACCOUNT FOR ASYMMETRY IN THE DISTRIBUTION OF EFFECT SIZES WHEN IDENTIFYING DIFFERENTIALLY EXPRESSED

3.1. Summary

A common statistical method used to analyze RNA-Seq data is Significance Analysis of Microarray with emphasis on RNA-Seq data (SAMseq). SAMseq is a nonparametric method that uses a resampling technique to account for differences in sequencing depths when identifying DE genes. Modifications of this method are made to take into account asymmetry in the distribution of the effect sizes by taking into account the sign of the test statistics. Through simulation studies, the proposed methods, compared with the traditional SAMseq method, provide better power for identifying truly DE genes while sufficiently controlling FDR in most settings. Illustration on the use of the proposed methods are made by reanalyzing RNA-Seq data from C57BL/6J (B6) and DBA/2J (D2) mouse strains samples.

3.2. Introduction

Sequencing approaches measure gene expressions as counts. The Poisson distribution has been the fundamental distribution used in modelling expression data (Audic and Claverie, 1997; Kal et al., 1999; Madden et al., 1997), and commonly applied to RNA-Seq data (Bullard et al., 2010; Marioni et al., 2008b). As an extension to the original SAM method (Tusher et al., 2001), Li and Tibshirani (2013) proposed a non-parametric approach known as Significance Analysis of Microarrays with emphasis on RNA-Seq data (SAMseq) to identify DE genes in RNA-Sequencing and other sequencing-based comparative genomic experiments. However, these tests are not free from error; thus, there is the risk of falsely identifying equivalently expressed (EE) genes as DE. In the Li and Tibshirani (2013) SAMseq procedure, they employ the use of a permutation plug-in method (Storey, 2002; Storey and Tibshirani, 2003; Tusher et al., 2001) to estimate the false discovery rate (Benjamini and Hochberg, 1995). This procedure uses permutations to generate the null distribution of the test statistic and estimate the false discovery rate (FDR) at a given cutoff point (C) as

$$F\hat{D}R_C = \hat{\pi}_0 \frac{\hat{V}}{\hat{R}}, \tag{3.1}$$

where $\hat{\pi}_0$ is the estimated proportion of null features in the population, $\hat{V}$ is the estimated number of false discoveries (i.e., genes that are EE but declared to be DE) when C is used as the cutoff point, and $\hat{R}$ is the estimated number of genes declared to be differentially expressed (DDE) when C is used as the cutoff point.

Ideally, researchers desire to identify all DE genes and no equivalently expressed (EE) genes between conditions (or classes) in a gene expression experiment. This is infeasible, however, so researchers seek to use the method that identifies the most DE genes while minimizing the number of EE genes that are declared DE. Identifying more DE genes (and fewer EE genes) allows researchers to more easily make important biological discoveries based on gene expression experiments. Thus, this propose to modify a commonly-used method to improve identification of DE genes while still adequately controlling false discovery rate (FDR).

In this chapter, our focus is on two class experiments. An example of a two class experiment data set is shown in Table 2. Suppose we obtain n_i RNA-Seq experiments for class i (i = 1, 2), and each experiment measures the expression levels of the same m genes on a subject. The data can then be represented as a $m \times (n_1 + n_2)$ matrix $\mathbf{G}$, whose element G_{ij} is the measure of expression Gene j in Experiment i, where $1 \le i \le n_i$, and $1 \le j \le m$.

Table 2. RNA-Seq data set for a two class experiment.

	Class 1				Class 2			
Gene	1	2	…	n_1	1	2	…	n_2
1	20	42	…	15	54	44	…	35
2	444	450	…	200	230	540	…	320
…	…	…	…	…	…	…	…	…
m	151	167	…	101	182	617	…	210

The SAMseq procedure does not explicitly take into account asymmetry in the distribution of the test statistics. Orr et al. (2014) showed in a two class gene expression experiments that taking into account asymmetry in the distribution of the test statistics when calculating q-values, another common method used to estimate false discovery rates (Storey, 2002), improved the identification of DE genes when asymmetry was apparent.

Motivated by the results of Orr et al. (2014) discussed in chapter 2, this research proposes two new methods that modifies the FDR estimation used in SAMseq to take into account such asymmetry. The first goal is to determine if taking into account this asymmetry when analyzing RNA expression data leads to an improvement over the traditional SAMseq method in the number of truly DE genes identified as differentially expressed, while still adequately controlling false discovery rate. The second goal is to compare the performance of the suitable proposed method to other commonly-used existing methods for identifying DE genes from RNA-Seq experiments. This is addressed in Chapter 4.

The rest of this chapter is organized as follows. In section 3.3; review of the SAMseq method for two class experiments and propose two methods that modifies the procedure used in estimating FDR. Section 3.4 describes and presents the results of simulation studies implemented to compare the performances of the proposed methods and traditional SAMseq method in terms of identification of differential expressed genes and FDR control. Section 3.5 presents analysis of a real RNA-Seq dataset using all methods from the simulation studies. All analyses are performed in R. Code from the samr package is used and modified to implement the proposed methods. Lastly, conclusions and recommendations are discussed in section 3.6.

3.3. Methods

Consider the problem of simultaneously testing multiple null hypotheses $H_1,\ldots,H_m$, where the j^{th} hypothesis is

$$H_j : \text{Gene } j \text{ is EE between the two classes.} \qquad (3.2)$$

Thus, if H_j is false, then gene j is said to be differentially expressed (DE). Moreover, if H_j is rejected, then gene j is declared to be differentially expressed. Ultimately, a researcher wants to determine which hypotheses should be rejected (i.e. determine which genes to declare to be DE) while controlling false discovery rate at a nominal level α.

In this section, an overview of the SAMseq method for estimating the FDR associated with each hypothesis $H_1,\ldots,H_m$ using two independent samples of RNA-Seq data. Additionally, proposed methods that modifies the FDR estimation used in SAMseq to account for asymmetry in the distribution of effect sizes.

3.3.1. Overview of SAMseq for two class unpaired comparison

Li and Tibshirani (2013) outlined the following steps for estimating FDR for a given cutoff Δ using two independent samples of RNA-Seq data;

(1) Using experiment 1 as the base level, estimate the sequencing depths for each experiment as

$$d_i = \frac{E\left(G_{ij}\right)}{E\left(G_{1j}\right)}; \qquad 1 \le i \le n,\ 1 \le j \le m\,, \tag{3.3}$$

where $E\left(G_{ij}\right)$ is the mean expression count for all genes in Experiment *i.* Note that this implies $d_1 = 1$.

(2) Resample S times from the data using the estimated depths $d_1,...,d_n$. The following steps outline the Poisson sampling strategy used;

a. Estimate the geometric mean $\overline{d}$ of the sequencing depths as

$$\overline{d} = \left(\prod_{i=1}^{n} d_i\right)^{\frac{1}{n}} \tag{3.4}$$

b. For each experiment *i*, the count is resampled as

$$G'_{ij} \sim Poisson\left(\frac{\overline{d}}{d_i} G_{ij}\right), \tag{3.5}$$

where G_{ij} is the read count for the j^{th} gene in experiment *i*.

c. A small random number is added to each count to account for ties between $G'_{1j}...,G'_{nj}$. Thus $G'_{ij} = G'_{ij} + \varepsilon_{ij}$ where ε_{ij} are independent identically distributed random variables generated from $Uniform\left(0, 0.1\right)$.

(3) Compute and order the test statistics on each resampled dataset. The Wilcoxon statistic for the j^{th} gene is calculated as

$$T_j^* = \frac{1}{S}\sum_{s=1}^{S}\left(\sum_{t\in C_1} R_{tj}\left(G'^s\right) - \frac{n_1\left(n+1\right)}{2}\right);\ t = 1, 2. \tag{3.6}$$

where C_t represents the subset of data from the t^{th} sample, G'^s represents the expression values for the s^{th} resampled data set, $R_{tj}\left(G'^s\right)$ is the rank of G'^s in $G_{1j},\ldots,G_{nj}$ and n_1 is the number of experiments in the first sample.

(4) Permute the read counts from the n experiments B times to obtain B permuted data sets. For the b^{th} permutation, compute test statistic $T_1^{*b},\ldots,T_m^{*b}$ based on the permuted data and order.

(5) Estimate the expected order statistic $\overline{T}_{(1)}^{*b},\ldots,\overline{T}_{(m)}^{*b}$ as

$$\overline{T}_j^{*b} = \frac{1}{B}\sum\nolimits_b T_{(j)}^{*b} \tag{3.7}$$

(6) For a given Δ; genes with positive test statistic $T_j^* \geq 0$ are called significant positive if $T_{(j)}^* - \overline{T}_{(j)}^{*b} > \Delta$ and genes with negative test statistic $T_j^* < 0$ are called significant negative if $\overline{T}_{(j)}^{*b} - T_{(j)}^* > \Delta$.

(7) Determine $cut_{up}\left(\Delta\right)$, the minimum value of the test statistics T_j^* among all significant positive genes, and $cut_{low}\left(\Delta\right)$, the maximum value of the test statistic T_j^* among all significant negative genes.

(8) Compute the number of falsely called (FC) genes, i.e. the number of EE genes that are called significant, among the b set of permutations as

$$FC^b\left(\Delta\right) = \sum_{j=1}^{m} I\left\{T_j^{*b} > cut_{up}\left(\Delta\right)\right\} + I\left\{T_j^{*b} < cut_{low}\left(\Delta\right)\right\} \tag{3.8}$$

(9) Estimate the proportion of true null genes π_0, in the data set as

$$\hat{\pi}_0 = \frac{\sum_j I\left\{T_j^* \in \left(q25, q75\right)\right\}}{0.5m} \tag{3.9}$$

where $q25$ and $q75$ are the 25th and 75th points of all permuted test statistics (among all B permutations). If the estimated proportion of true null genes is greater than one, set the proportion of true null genes to be equal to one.

(10) Compute the false discovery rate as

$$FDR\left(\Delta\right) = \frac{\hat{\pi}_0 medFC\left(\Delta\right)}{Number\ of\ significant\ genes\left(\Delta\right)} \tag{3.10}$$

where $medFC\left(\Delta\right)$ is the median number of falsely called genes among the B permuted datasets. Starting in Chapter 3, we will refer to this method used to estimate FDR as the "traditional method".

3.3.2. Proposed methods for estimating FDR

The method described in section 3.3.1 does not account for asymmetry in the distribution of the test statistics, if such asymmetry exists. Orr et al. (2014) showed that taking into account apparent asymmetry in the test statistics by modifying Storey's q-value results in higher power for detecting DE genes when such asymmetry exists. Using this as motivation, this research proposes two methods that modify the FDR estimation of the SAMseq method by taking into account the asymmetry of the test statistics.

3.3.2.1. Proposed method I

For proposed method I, steps (1) through (7) of the SAMseq procedure outlined in section 3.3.1. is used. To estimate FDR, begin by dividing the test statistics into two groups based on sign. For genes with positive test statistics, estimate the number of falsely called positive genes for each permuted data set among the B set of permutations as

$$FC^b + (\Delta) = \sum_{j=1}^{m} I\left\{T_j^{*b} > cut_{up}(\Delta)\right\}, \tag{3.11}$$

and for genes with negative test statistics, estimate the number of falsely negatively called genes among the *B* set of permutations as

$$FC^b - (\Delta) = \sum_{j=1}^{m} I\left\{T_j^{*b} < cut_{low}(\Delta)\right\}. \tag{3.12}$$

Next, calculate the median number of falsely positively called genes as

$$medFC + (\Delta) = median\left\{FC^b + (\Delta)\right\} \tag{3.13}$$

and the median number of falsely negatively called genes as

$$medFC - (\Delta) = median\left\{FC^b - (\Delta)\right\}. \tag{3.14}$$

The proportion of EE genes π_0 is estimated exactly as in equation (3.9). Then estimate the proportion of EE genes among genes with positive test statistics, that is, $T_{(j)}^{*} \geq 0$ as

$$\hat{\pi}_0^{+} = \frac{m\,\hat{\pi}_0/2}{m^{+}}, \tag{3.15}$$

where *m* is the total number of genes in an experiment and, m^{+} is the number of genes with $T_{(j)}^{*} \geq 0$. Similarly, estimate the proportion of EE genes among genes with negative test statistics, that is, $T_{(j)}^{*} < 0$ as

$$\hat{\pi}_0^{-} = \frac{m\,\hat{\pi}_0/2}{m^{-}}, \tag{3.16}$$

where m^{-} is the number of genes with $T_{(j)}^{*} < 0$.

The estimates in (3.15) and (3.16) are based on the assumption that the asymmetry present in the distribution of the test statistics is due to asymmetry in the distribution of the effect sizes of DE genes and that EE genes have test statistics that are symmetric (or very close to symmetric) around zero. Thus, a researcher expect that the number of EE genes with positive test statistics is equal to the number of EE genes with negative test statistics, on average, and this number is estimated to be $\hat{\pi}_0/2$.

Lastly for a given Δ, estimate FDR as

$$FDR(\Delta) = \frac{\hat{\pi}_0^+ medFC(+) + \hat{\pi}_0^- medFC(-)}{Number\ of\ significant\ genes(\Delta)}. \tag{3.17}$$

The estimation of FDR in (3.17) modifies the numerator in (3.10) by taking into account the asymmetry in the test statistics but maintains the same cutoff (Δ) for both positive and negative test statistics.

3.3.2.2. Proposed method II

For the proposed method II, steps (1) through (5) of the SAMseq procedure in section 3.3.1 and estimation of the proportion of equally expressed genes, $\hat{\pi}_0$, in the data set as described in equation (3.9). Next, divide the test statistics into two groups based on the sign of the test statistics and estimate FDR separately for genes with positive test statistics and genes with negative test statistics. The FDR estimation for genes with positive test statistics, i.e., $T_{(j)}^* \geq 0$; for a given value Δ^+, a gene is significant positive if $T_{(j)}^* - \overline{T}_{(j)}^{*b} > \Delta^+$. Next, estimate $cut_{up}(\Delta^+)$, that is, the minimum value of the test statistics $T_{(j)}^*$ among all significant positive genes. Given *B* sets of permuted and ordered test statistics; calculate the number of falsely positively called genes, i.e. the number of EE genes among significant positive genes, as

$$FC^b(\Delta^+) = \sum_{j=1}^{m} I\left\{T_j^{*b} > cut_{up}(\Delta^+)\right\} \tag{3.18}$$

and estimate the median number of falsely positively called genes as

$$medFC\left(\Delta^{+}\right)=median\left\{FC^{b}\left(\Delta^{+}\right)\right\}\text{, i.e, } median\left\{FC^{b}\left(\Delta^{+}\right);\;\; b=1,2,\ldots,B\right\}. \tag{3.19}$$

The proportion of genes with positive test statistics $T_{(j)}^{*}\geq 0$ that are EE is estimated as

$$\hat{\pi}_{0}^{+}=\frac{m\,\hat{\pi}_{0}/2}{m^{+}}. \tag{3.20}$$

where *m* is the total number of genes in an experiment and, m^{+} is the number of genes with $T_{(j)}^{*}\geq 0$.

For a given Δ^{+}, estimate the FDR for genes with positive test statistics as

$$FDR\left(\Delta^{+}\right)=\frac{\hat{\pi}_{0}^{+}medFC\left(\Delta^{+}\right)}{Number\ of\ significant\ positive\ genes\left(\Delta^{+}\right)} \tag{3.21}$$

For genes with negative test statistics, i.e., $T_{(j)}^{*}<0$; a gene is significant negative if $\overline{T}_{(j)}^{*b}-T_{(j)}^{*}>\Delta^{-}$.

Next, $cut_{low}\left(\Delta^{-}\right)$ the maximum value of the test statistics $T_{(j)}^{*}$ among all significant negative genes, is determined. For each of the *B* sets of permuted and ordered test statistics, calculate the number of falsely negatively called genes i.e. the number of EE genes among significant negative genes as

$$FC^{b}\left(\Delta^{-}\right)=\sum_{j=1}^{m}I\left\{T_{j}^{*b}<cut_{low}\left(\Delta^{-}\right)\right\}, \tag{3.22}$$

and estimate the median number of falsely negatively called genes as

$$medFC\left(\Delta^{-}\right)=median\left\{FC^{b}\left(\Delta^{-}\right)\right\}\text{, i.e., } median\left\{FC^{b}\left(\Delta^{-}\right);\;\; b=1,2,\ldots,B\right\} \tag{3.23}$$

Then estimate the proportion of genes with $T_{(j)}^{*}<0$ that are EE as

$$\hat{\pi}_{0}^{-}=\frac{m\,\hat{\pi}_{0}/2}{m^{-}}, \tag{3.24}$$

where m^- is the number of genes with $T^*_{(j)} < 0$, and estimate the FDR for genes with negative test statistics as

$$FDR\left(\Delta^-\right) = \frac{\hat{\pi}_0^- medFC\left(\Delta^-\right)}{Number\ of\ significant\ negaitive\ genes\left(\Delta^-\right)}. \tag{3.25}$$

The estimates in (3.20) and (3.24) are based on the assumption that the asymmetry present in the distribution of the test statistics is due to asymmetry in the distribution of the effect sizes of DE genes and that EE genes have test statistics that are symmetric (or very close to symmetric) around zero. Thus, the expected the number of EE genes with positive test statistics is equal to the number of EE genes with negative test statistics, on average, and this number is estimated to be $\hat{\pi}_0 / 2$. The estimation of FDR in (3.21) and (3.25) modifies the numerator in (3.10) by taking into account the asymmetry in the test statistics and uses different delta values for positive and negative test statistics.

3.4. Simulation studies

In order to evaluate the performance of the proposed methods compared to the traditional method (Li andTibshirani, 2013) for estimating false discovery rate; data sets with Poisson distributed gene counts were randomly generated. For each data set, gene counts were randomly generated for m = 10,000 genes in two experiments. For gene j in experiment i, the gene count was generated as

$$G_{ij} \sim Poisson\left(\mu_{ij}\right) \tag{3.26}$$

and

$$\log \mu_{ij} = \log d_i + \log \upsilon_j + \gamma_j I_{(i \in C_2)}, \tag{3.27}$$

where d_i is the sequencing depth of experiment *i*, υ_j is the expression level of gene *j* in the first group, and γ_j represents the difference in gene expression between the two experiments for gene *j* if it is differentially expressed. Using procedures implemented by Li *et al.* (2012),

$$d_i \sim \exp\left(uniform\left(4,6\right)\right), \tag{3.28}$$

is simulated so that the total number of reads are similar to real RNA-seq experiments;

$$\upsilon_j = \frac{G_j}{\frac{1}{m}\sum_1^m G_j}, \tag{3.29}$$

is simulated so that gene expression levels are similar to a real RNA-seq data set (Marioni et al., 2008b);

$$\gamma_j \sim \left|N\left(0,1\right)\right|, \tag{3.30}$$

for upregulated genes, and for down regulated genes

$$\gamma_j \sim -\left|N\left(0,1\right)\right|, \tag{3.31}$$

are simulated so that the average fold change for differentially expressed genes is about 2.7. For EE genes,

$$\gamma_j = 0 \tag{3.32}$$

To create difference simulation settings, simulated data sets with four different sample sizes, $n = \{4,6,10,12\}$ and four different values for the number of EE genes, $m_0 = \{5000,7000,9000,9500\}$ are used. To simulate asymmetry, five set of values representing the proportion of DE genes that are upregulated and downregulated were used: $\pi_1 = \left(0.5,0.5\right)$, $\pi_2 = \left(0.7,0.3\right)$, $\pi_3 = \left(0.8,0.2\right)$, $\pi_3 = \left(0.9,0.1\right)$, and $\pi_5 = \left(0.95,0.05\right)$. For instance, in settings where $\pi_3 = \left(0.8,0.2\right)$ is used, 0.8 represent the proportion of DE genes that are upregulated and

0.2 represent the proportion of DE genes that are downregulated in the data set. This results in eighty different simulation settings.

3.4.1. Results

For each simulation setting, 100 data sets were randomly generated. For each data set, all three methods (proposed method I, proposed method II and traditional method) were used to estimate the FDR for each gene to identify DE genes. For a given delta value, FDRs were calculated using all methods. Although delta values are usually user defined, a set of delta values was sequenced and the value of delta was chosen that corresponded to an estimated FDR closest to but less than 0.05 (or 5%).

Controlling FDR at the 5% significance level, *S* (the number of DE genes DDE) was determined for each data set. To determine if each method controlled FDR at the 5% significance level, the observed FDR, V/R (the proportion of EE genes among all DDE genes) was calculated for each data set. If no genes were DDE for a particular data set, V/R was set to zero. For each simulation setting, paired *t*-tests were performed to test the difference in the mean *S* of proposed method I and the traditional method, proposed method II and the traditional method, proposed method I and proposed method II. If the test between these comparisons were significant at a type I error rate of 5%, then the higher mean *S* is shown in bolded font. If a test between proposed method I and proposed method II was significant at a type I error rate of 5% with the proposed method II outperforming the proposed method I, the higher mean *S* is underlined. Table 3 and Table 4 below presents the mean S and mean V/R for each simulation setting, respectively. The corresponding standard errors for the mean S and mean V/R are reported in parentheses.

As expected, the power to detect DE genes increased as the number of EE genes decreased, that is, the number of DE genes (m_0) increased. Also, the power to identify DE genes increased as the sample size increased.

Pertaining to the initial goal of this research, the traditional method did not outperform the proposed method I and II in any of the simulation settings in terms of mean S, as seen in Table 3. Proposed method I performed better than the traditional method in 59 of the 80 simulation settings with regard to mean S (10 of 20 settings with $n = 4$, 16 of 20 settings with $n = 6$, 16 of 20 settings with $n = 10$, and 17 of 20 settings with $n = 12$). The proposed method II performed better than the traditional method in 69 of the 80 simulations, including all settings with $n = 6$, 18 of 20 settings with $n = 10$; 19 of 20 settings with $n = 12$, and 12 of 20 settings with $n = 4$. Furthermore, proposed method II performed better than proposed method I in 62 of 80 settings in terms of mean S (6 of 20 settings with $n = 4$, 20 of 20 settings with $n = 6$, 18 of 20 settings with $n = 10$, and 18 of 20 settings with $n = 12$). Although a higher value of mean S was observed in the traditional method compared to the proposed methods I and II in the setting where sample size $n = 10$, $m_0 = 9000$, and π_1; this difference was not significant. Also higher values of mean S was observed in proposed method II compared to proposed method I, but there were no significant differences between these two methods in 3 of 80 settings. Apart from these settings, a higher value of mean S was observed using the traditional method compared to proposed method I, but not proposed method II in 9 of 80 settings, but there was no significant difference in mean S at 5% significance between the traditional method and proposed method I.

As shown in Table 4, the observed FDR (mean V/R) was comparable among the proposed methods and traditional method for each simulation setting, with levels elevated above

5% for the simulation settings with the smallest sample size (*n* = 4). In the simulation settings with all other sample sizes, the observed FDR was controlled at, or close to, 5% for all methods.

Table 3. The mean S for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting.

				Mean S		
				Traditional	**Proposed**	
n	m_0	**DE**	π_i		**I**	**II**
4	**5000**	**5000**	π_1	603.770 (5.779)	584.030 (5.768)	**665.040 (4.746)**
			π_2	519.010 (2.914)	**569.940 (4.511)**	**671.570 (7.292)**
			π_3	558.070 (4.439)	**610.600 (3.851)**	**613.570 (3.819)**
			π_4	728.700 (4.714)	**801.540 (4.091)**	**802.560 (4.063)**
			π_5	700.850 (4.471)	**792.640 (4.380)**	**792.640 (4.380)**
	7000	**3000**	π_1	100.820 (4.425)	94.700 (4.584)	**120.560 (6.076)**
			π_2	183.910 (4.296)	**201.310 (2.423)**	**208.450 (2.867)**
			π_3	225.540 (2.825)	**250.930 (3.117)**	**250.930 (3.117)**
			π_4	254.060 (2.961)	**300.450 (2.617)**	**300.450 (2.617)**
			π_5	262.480 (3.231)	**324.180 (3.857)**	**324.180 (3.857)**
	9000	**1000**	π_1	0.770 (0.384)	0.330 (0.233)	<0.001 (<0.001)
			π_2	<0.001 (<0.001)	0.570 (0.412)	0.570 (0.412)
			π_3	4.200 (0.993)	4.650 (1.072)	4.650 (1.072)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If one method has a significant higher mean *S* compared to the other two methods, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 3. The mean S for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S		
				Traditional	**Proposed**	
n	***m_0***	**DE**	**π_i**		**I**	**II**
4	**9000**	**1000**	**π_4**	10.050 (1.693)	**23.180 (2.111)**	**23.180 (2.111)**
			π_5	26.450 (2.433)	**31.660 (2.404)**	**31.660 (2.404)**
	9500	**500**	**π_1**	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
			π_2	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
			π_3	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
			π_4	<0.001 (<0.001)	0.330 (0.237)	0.330 (0.237)
			π_5	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
6	**5000**	**5000**	**π_1**	3142.390 (2.816)	**3145.260 (2.676)**	**3167.580 2.541)**
			π_2	2455.030 (4.608)	**2543.140 (7.385)**	**3292.600 (2.541)**
			π_3	2643.070 (2.687)	**2674.230 (2.613)**	**3210.310 (2.884)**
			π_4	3144.200 (2.161)	**3186.670 (2.491)**	**3458.040 (2.710)**
			π_5	3281.200 (2.506)	**3321.780 (2.244)**	**3439.340 (4.547)**
	7000	**3000**	**π_1**	1278.380 19.220)	1281.960 (19.143)	**1540.420 (2.121)**
			π_2	1399.460 (1.775)	**1425.710 (2.871)**	**1860.730 (1.875)**
			π_3	1647.370 (1.580)	**1662.400 (1.599)**	**1987.230 (1.716)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If one method has a significant higher mean *S* compared to the other two methods, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 3. The mean S for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S		
				Traditional	**Proposed**	
n	***m*₀**	**DE**	π_i		**I**	**II**
6	**7000**	**3000**	π_4	1699.210 (1.857)	**1728.290 (1.793)**	**1830.980 (4.729)**
			π_5	1919.720 (1.744)	**1951.730 (1.741)**	**1976.980 (2.299)**
	9000	**1000**	π_1	515.200 (7.394)	517.260 (7.208)	**535.970 (5.080)**
			π_2	393.760 (7.155)	**407.070 (4.239)**	**517.720 (6.150)**
			π_3	505.130 (0.777)	**507.830 (0.753)**	**578.540 (3.576)**
			π_4	525.390 (3.935)	**527.950 (3.962)**	**542.750 (4.241)**
			π_5	479.170 (0.955)	**483.380 (0.965)**	**491.180 (1.252)**
	9500	**500**	π_1	189.030 (1.222)	**193.190 (2.239)**	**231.910 (6.203)**
			π_2	159.530 (8.129)	**172.280 (7.593)**	**218.040 (8.390)**
			π_3	199.210 (7.111)	195.430 (7.370)	**212.680 (7.584)**
			π_4	253.200 (3.600)	253.260 (3.600)	**259.150 (3.650)**
			π_5	244.320 (6.124)	**260.470 (3.373)**	**262.940 (3.350)**
10	**5000**	**5000**	π_1	3333.470 (2.514)	3332.570 (2.516)	**3338.570 (2.454)**
			π_2	3487.460 (2.623)	**3511.980 (2.715)**	**3555.450 (2.499)**
			π_3	3566.390 (2.649)	**3609.980 (2.604)**	**3668.730 (2.497)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If one method has a significant higher mean *S* compared to the other two methods, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 3. The mean S for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S		
				Traditional	**Proposed**	
n	***m*₀**	**DE**	π_i		**I**	**II**
10	**5000**	**5000**	π_4	3476.510 (6.919)	**3558.420 (4.318)**	**3621.910 (2.080)**
			π_5	3682.050 (4.375)	**3758.830 (5.453)**	**3831.440 (2.445)**
	7000	**3000**	π_1	2046.110 (1.600)	2045.460 (1.593)	**2049.050 (1.542)**
			π_2	1915.780 (1.747)	**1922.750 (1.706)**	**1944.820 (1.722)**
			π_3	1961.270 (1.615)	**1979.750 (1.569)**	**2000.900 (1.406)**
			π_4	2195.010 (4.836)	**2250.420 (1.377)**	**2276.420 (1.377)**
			π_5	2162.030 (1.841)	**2198.260 (2.025)**	**2269.640 (1.555)**
	9000	**1000**	π_1	625.040 (0.764)	624.390 (0.737)	624.620 (0.762)
			π_2	600.960 (0.968)	600.170 (0.986)	**608.800 (1.020)**
			π_3	653.700 (0.794)	**656.220 (0.762)**	**662.280 (0.768)**
			π_4	588.770 (0.953)	**589.820 (0.909)**	**621.650 (0.902)**
			π_5	681.500 (0.744)	**685.670 (0.730)**	**707.460 (0.690)**
	9500	**500**	π_1	293.450 (0.541)	**293.690 (0.543)**	293.430 (0.555)
			π_2	286.600 (0.564)	**287.770 (0.557)**	**289.400 (0.518)**
			π_3	307.240 (0.557)	**307.890 (0.563)**	**309.700 (0.553)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If one method has a significant higher mean *S* compared to the other two methods, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 3. The mean S for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S		
				Traditional	**Proposed**	
n	***m*₀**	**DE**	π_i		**I**	**II**
10	**9500**	**500**	π_4	315.450 (0.762)	**317.470 (0.681)**	**319.260 (0.582)**
			π_5	317.030 (0.505)	**317.130 (0.504)**	**326.800 (0.534)**
12	**5000**	**5000**	π_1	3621.600 (3.078)	**3628.480 (2.681)**	**3631.800 (2.681)**
			π_2	3408.150 (3.805)	**3443.260 (3.507)**	**3484.980 (3.192)**
			π_3	3462.340 (3.433)	**3500.930 (3.541)**	**3550.160 (3.049)**
			π_4	3597.780 (2.699)	**3669.350 (3.075)**	**3699.720 (2.628)**
			π_5	3694.540 (3.381)	**3748.770 (2.958)**	**3776.480 (2.807)**
	7000	**3000**	π_1	2021.820 (1.691)	2022.220 (1.636)	**2026.860 (1.689)**
			π_2	2084.570 (1.540)	**2098.030 (1.507)**	**2113.840 (1.458)**
			π_3	2115.730 (1.570)	**2131.110 (1.585)**	**2150.520 (1.482)**
			π_4	2211.550 (1.612)	**2237.970 (1.733)**	**2254.140 (1.674)**
			π_5	2240.620 (1.916)	**2279.460 (1.620)**	**2289.900 (1.632)**
	9000	**1000**	π_1	655.090 (0.789)	655.010 (0.788)	655.110 (0.780)
			π_2	679.660 (0.727)	**680.190 (0.729)**	**684.700 (0.732)**
			π_3	646.790 (0.914)	**648.140 (0.893)**	**653.430 (0.849)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If one method has a significant higher mean *S* compared to the other two methods, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 3. The mean S for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S		
				Traditional	**Proposed**	
n	***m_0***	**DE**	**π_i**		**I**	**II**
12	**9000**	**1000**	**π_4**	728.650 (0.622)	**731.510 (0.624)**	**735.200 (0.609)**
			π_5	702.520 (0.745)	**705.240 (0.781)**	**708.540 (0.778)**
	9500	**500**	**π_1**	310.950 (0.493)	**311.390 (0.488)**	**311.570 (0.511)**
			π_2	325.440 (0.437)	324.910 (0.445)	**326.610 (0.422)**
			π_3	300.360 (0.475)	**300.660 (0.484)**	**301.850 (0.436)**
			π_4	323.550 (0.493)	**324.130 (0.490)**	**325.420 (0.493)**
			π_5	320.840 (0.472)	**321.550 (0.472)**	**323.060 (0.498)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If one method has a significant higher mean *S* compared to the other two methods, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 4. The mean V/R for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting.

				Mean V/R		
				Traditional	**Proposed**	
n	***m_0***	**DE**	**π_i**		**I**	**II**
4	**5000**	**5000**	**π_1**	0.193 (0.002)	0.190 (0.002)	0.161 (0.002)
			π_2	0.121 (0.002)	0.140 (0.002)	0.142 (0.002)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 4. The mean V/R for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R		
				Traditional	**Proposed**	
n	***m*₀**	**DE**	$\boldsymbol{\pi_i}$		**I**	**II**
4	**5000**	**5000**	$\boldsymbol{\pi_3}$	0.102 (0.002)	0.116 (0.001)	0.117 (0.001)
			$\boldsymbol{\pi_4}$	0.084 (0.001)	0.100 (0.001)	0.100 (0.001)
			$\boldsymbol{\pi_5}$	0.079 (0.001)	0.101 (0.001)	0.101 (0.001)
	7000	**3000**	$\boldsymbol{\pi_1}$	0.176 (0.007)	0.166 (0.008)	0.154 (0.006)
			$\boldsymbol{\pi_2}$	0.143 (0.004)	0.157 (0.003)	0.158 (0.003)
			$\boldsymbol{\pi_3}$	0.150 (0.003)	0.173 (0.003)	0.173 (0.003)
			$\boldsymbol{\pi_4}$	0.145 (0.003)	0.177 (0.003)	0.177 (0.003)
			$\boldsymbol{\pi_5}$	0.122 (0.002)	0.159 (0.003)	0.159 (0.003)
	9000	**1000**	$\boldsymbol{\pi_1}$	0.014 (0.007)	0.008 (0.006)	<0.001 (<0.001)
			$\boldsymbol{\pi_2}$	<0.001 (<0.001)	0.005 (0.004)	0.005 (0.004)
			$\boldsymbol{\pi_3}$	0.044 (0.010)	0.046 (0.011)	0.046 (0.011)
			$\boldsymbol{\pi_4}$	0.089 (0.015)	0.178 (0.016)	0.178 (0.016)
			$\boldsymbol{\pi_5}$	0.165 (0.015)	0.195 (0.015)	0.195 (0.015)
	9500	**500**	$\boldsymbol{\pi_1}$	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
			$\boldsymbol{\pi_2}$	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
			$\boldsymbol{\pi_3}$	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 4. The mean V/R for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R		
				Traditional	**Proposed**	
n	***m*₀**	**DE**	π_i		**I**	**II**
4	**9500**	**500**	π_4	<0.001 (<0.001)	0.010 (0.007)	0.010 (0.007)
			π_5	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
6	**5000**	**5000**	π_1	0.048 (<0.001)	0.048 (<0.001)	0.047 (<0.001)
			π_2	0.062 (0.001)	0.73 (0.001)	0.069 (0.001)
			π_3	0.024 (<0.001)	0.028 (<0.001)	0.034 (<0.001)
			π_4	0.020 (<0.001)	0.025 (<0.001)	0.034 (<0.001)
			π_5	0.017 (<0.001)	0.020 (<0.001)	0.036 (<0.001)
	7000	**3000**	π_1	0.046 (0.001)	0.045 (0.001)	0.038 (0.001)
			π_2	0.043 (0.001)	0.054 (0.001)	0.044 (<0.001)
			π_3	0.037 (<0.001)	0.043 (0.001)	0.043 (0.001)
			π_4	0.043 (0.001)	0.052 (0.001)	0.051 (0.001)
			π_5	0.038 (0.001)	0.047 (0.001)	0.047 (0.001)
	9000	**1000**	π_1	0.067 (0.002)	0.067 (0.002)	0.063 (0.001)
			π_2	0.042 (0.002)	0.041 (0.001)	0.040 (0.001)
			π_3	0.040 (0.001)	0.044 (0.001)	0.044 (0.001)
			π_4	0.034 (0.001)	0.036 (0.001)	0.038 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 4. The mean V/R for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R		
				Traditional	**Proposed**	
n	***m*₀**	**DE**	$\boldsymbol{\pi_i}$		**I**	**II**
6	**9000**	**1000**	$\boldsymbol{\pi_5}$	0.040 (0.001)	0.044 (0.001)	0.044 (0.001)
	9500	**500**	$\boldsymbol{\pi_1}$	0.052 (0.002)	0.052 (0.002)	0.051 (0.003)
			$\boldsymbol{\pi_2}$	0.078 (0.006)	0.073 (0.005)	0.064 (0.004)
			$\boldsymbol{\pi_3}$	0.061 (0.005)	0.062 (0.005)	0.081 (0.005)
			$\boldsymbol{\pi_4}$	0.049 (0.003)	0.049 (0.003)	0.058 (0.003)
			$\boldsymbol{\pi_5}$	0.059 (0.004)	0.057 (0.003)	0.058 (0.002)
10	**5000**	**5000**	$\boldsymbol{\pi_1}$	0.047 (<0.001)	0.047 (<0.001)	0.047 (<0.001)
			$\boldsymbol{\pi_2}$	0.040 (<0.001)	0.043 (<0.001)	0.047 (<0.001)
			$\boldsymbol{\pi_3}$	0.035 (<0.001)	0.040 (<0.001)	0.048 (<0.001)
			$\boldsymbol{\pi_4}$	0.027 (<0.001)	0.032 (<0.001)	0.046 (<0.001)
			$\boldsymbol{\pi_5}$	0.029 (<0.001)	0.035 (<0.001)	0.089 (0.001)
	7000	**3000**	$\boldsymbol{\pi_1}$	0.048 (0.001)	0.048 (0.001)	0.049 (0.001)
			$\boldsymbol{\pi_2}$	0.044 (<0.001)	0.047 (0.001)	0.048 (0.001)
			$\boldsymbol{\pi_3}$	0.043 (0.001)	0.048 (0.001)	0.048 (0.001)
			$\boldsymbol{\pi_4}$	0.045 (<0.001)	0.053 (0.001)	0.052 (0.001)
			$\boldsymbol{\pi_5}$	0.045 (<0.001)	0.056 (0.001)	0.055 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 4. The mean V/R for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R		
				Traditional	**Proposed**	
n	m_0	**DE**	π_i		**I**	**II**
10	**9000**	**1000**	π_1	0.045 (0.001)	0.044 (0.001)	0.044 (0.001)
			π_2	0.044 (0.001)	0.043 (0.001)	0.048 (0.001)
			π_3	0.047 (0.001)	0.050 (0.001)	0.047 (0.001)
			π_4	0.040 (0.001)	0.041 (0.001)	0.041 (0.001)
			π_5	0.045 (0.001)	0.049 (0.001)	0.048 (0.001)
	9500	**500**	π_1	0.080 (0.002)	0.080 (0.002)	0.077 (0.002)
			π_2	0.061 (0.001)	0.065 (0.001)	0.059 (0.001)
			π_3	0.043 (0.001)	0.045 (0.001)	0.043 (0.001)
			π_4	0.048 (0.001)	0.052 (0.001)	0.050 (0.001)
			π_5	0.038 (0.001)	0.039 (0.001)	0.040 (0.001)
12	**5000**	**5000**	π_1	0.034 (0.001)	0.035 (<0.001)	0.035 (<0.001)
			π_2	0.036 (0.001)	0.040 (0.001)	0.042 (0.001)
			π_3	0.028 (<0.001)	0.033 (<0.001)	0.040 (<0.001)
			π_4	0.035 (<0.001)	0.044 (<0.001)	0.053 (0.001)
			π_5	0.025 (<0.001)	0.030 (<0.001)	0.053 (0.001)
	7000	**3000**	π_1	0.045 (<0.001)	0.045 (<0.001)	0.046 (<0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 4. The mean V/R for the proposed and traditional FDR methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R		
				Traditional	**Proposed**	
n	m_0	**DE**	π_i		**I**	**II**
12	**7000**	**3000**	π_2	0.050 (0.001)	0.055 (0.001)	0.053 (0.001)
			π_3	0.039 (<0.001)	0.044 (0.001)	0.045 (0.001)
			π_4	0.046 (<0.001)	0.057 (0.001)	0.056 (<0.001)
			π_5	0.054 (0.001)	0.068 (0.001)	0.066 (0.001)
	9000	**1000**	π_1	0.049 (0.001)	0.049 (0.001)	0.049 (0.001)
			π_2	0.044 (0.001)	0.044 (0.001)	0.045 (0.001)
			π_3	0.044 (0.001)	0.046 (0.001)	0.045 (0.001)
			π_4	0.049 (0.001)	0.054 (0.001)	0.053 (0.001)
			π_5	0.045 (0.001)	0.049 (0.001)	0.049 (0.001)
	9500	**500**	π_1	0.060 (0.001)	0.061 (0.001)	0.060 (0.001)
			π_2	0.048 (0.001)	0.046 (0.001)	0.053 (0.001)
			π_3	0.042 (0.001)	0.043 (0.001)	0.042 (0.001)
			π_4	0.041 (0.001)	0.043 (0.001)	0.043 (0.001)
			π_5	0.046 (0.001)	0.048 (0.001)	0.048 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

3.5. Real data analysis

In this section, RNA-Seq data from a real gene expression experiment described by Bottomly et al. (2011) using both the proposed methods and traditional (SAMseq) methods is analyzed. Using the Illumina GAIIx sequencing platform, the experiment was performed to evaluate gene expression in C57BL/6J (B6) and DBA/2J (D2) mouse striatum using RNA-Seq and microarrays. For the analysis, the focus is on the RNA-Seq data. There were two classes (B6 and D2); with a total of $n = 21$ samples, $n_1 = 10$ B6 samples and $n_2 = 11$ D2 samples. The data set contains 36,536 genes, with many of the genes not having any reads. These genes were removed, and the remaining $m = 13{,}932$ were analyzed. The raw data set is named after the first author of the paper and is available from ReCount project (Frazee et al., 2011) with an identifier "bottomly". Figure 2 below shows the distribution of the test statistic for the genes analyzed.

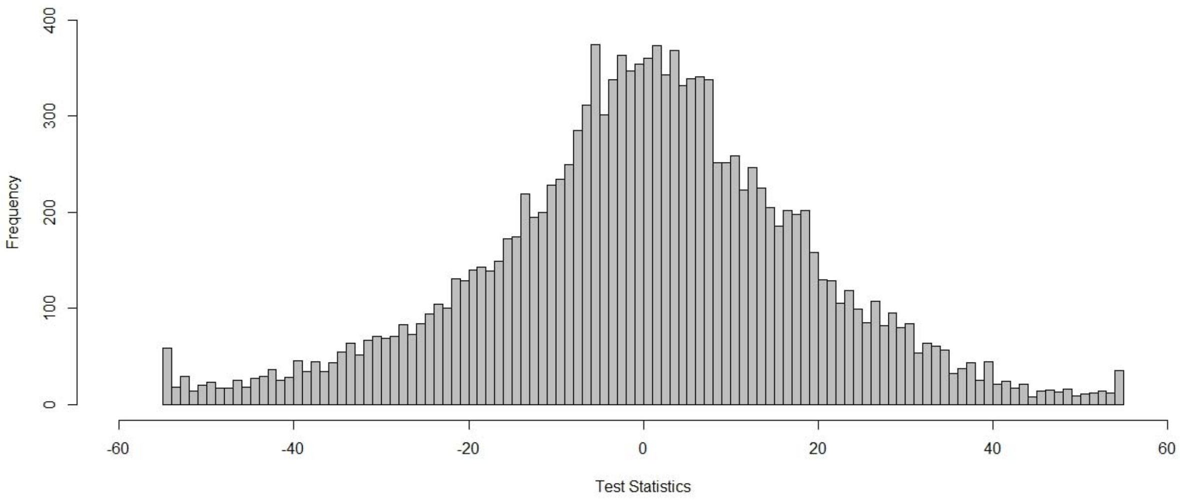

Figure 2. Histogram of the test statistic from the experiment described by Bottomly et al. (2011) using SAMseq two class unpaired test statistics, to compare RNA expression levels between B6 and D2 samples.

Although, the histogram of the test statistics from this experiment does not clearly indicate asymmetry in the distribution of test statistics; there are more genes with positive test statistics than negative test statistics. Precisely, there are $m^{+} = 7190$ genes with positive test statistics and $m^{-} = 6742$ genes with negative test statistics.

Using the method described in section 3.3.1 for estimating the proportion of EE genes π_0, $\hat{\pi}_0 = 0.7182$. Thus, the estimated number of EE genes was $\hat{m}_0 = 10006$. Since the expected EE genes should have an equal number of both positive and negative test statistics, then the estimate $\hat{m}_0/2 = 5003$ EE genes with positive test statistics and $\hat{m}_0/2 = 5003$ EE genes with negative test statistics. Using these estimates, estimate the number of DE genes with positive effect sizes as 7190 – 5003 = 2187 genes, and the number of DE genes negative effect sizes as 6742 – 5003 = 1739 genes. This results in an estimate of 56% of DE having positive effect sizes and 44% having negative effect sizes.

The number of genes declared to be DE using proposed method I, proposed method II and the traditional method while controlling FDR at 5% are summarized in Figure 3. There were 1868 genes that were DDE by all three methods. An additional 47 genes were DDE by the proposed method I and the traditional method, but not the proposed method II. Finally, there are 70 additional genes DDE by only proposed method II and 8 genes DDE by only the traditional method. Therefore, proposed method II declared the most genes to be DE, followed by the traditional method and then proposed method I. This is not surprising based on the results from the simulation studies in section 3.4.

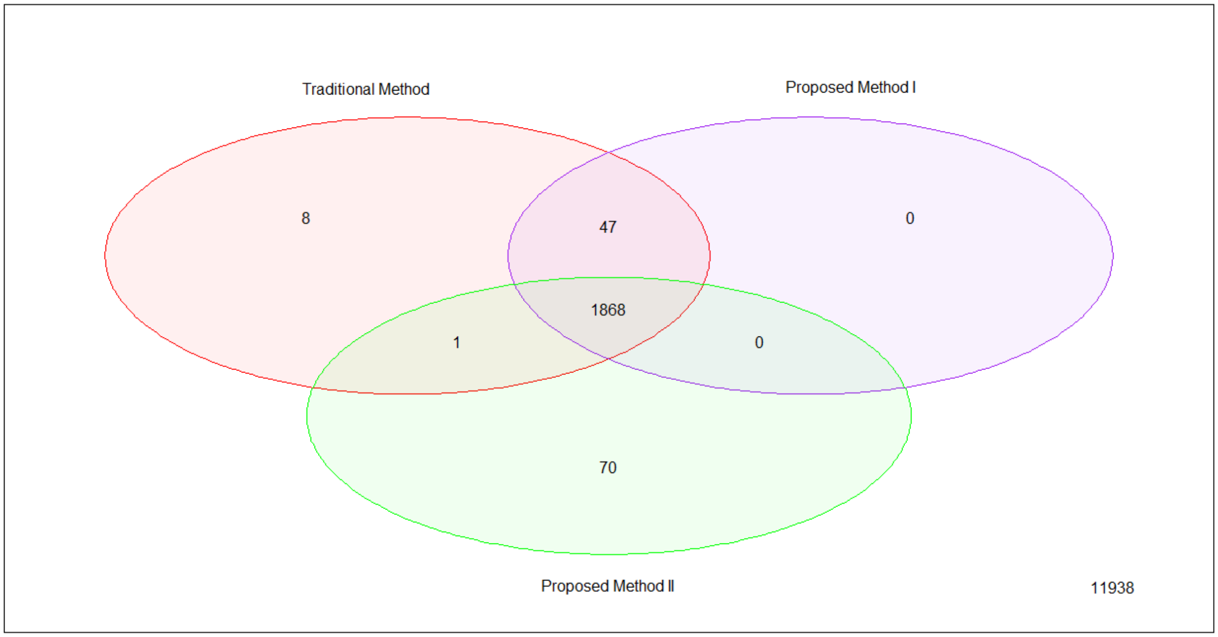

Figure 3. Venn diagram of genes declared to be DE for the proposed method I, proposed method II and traditional method.

Because this analysis was performed on a real, not simulated, data set, it cannot be determined which genes are EE and which are DE. Thus, evaluating the true FDR associated with each method is impossible. However, because the sample size for each class is relatively large with a small degree of asymmetry, the FDR is being adequately controlled at 5% based on the results of the simulation study in section 3.4.

3.6. Discussion

The proposed methods for estimating FDR, when there exists asymmetry in the distribution of the test statistics, has observed advantages over the traditional method. Proposed methods I and II were never outperformed by the traditional method in terms of identifying DE genes in the simulation studies and outperformed the traditional method in almost all settings where asymmetry was present. The proposed methods also adequately controlled FDR at 5% in most simulation settings with the exception of settings with $n = 4$. The power for detecting DE genes was also low when $n = 4$. Thus, the use of the proposed methods or traditional method for estimating FDR when the sample size is very small is not recommended. This is consistent with recommendations made by Li and Tibshirani (2013). Additionally, proposed method II performed better than proposed method I and the traditional method in most settings.

Using real RNA-Seq data, proposed method II declared more genes to be DE than proposed method I and the traditional method at 5% significance level, which is consistent with the simulation results.

Based on the results from the simulation studies and real data analyses, the proposed methods should be used to analyze experiments with sample sizes of at least 6 when there exists asymmetry in the distribution of the test statistics. Proposed method II is more preferable than proposed method I.

Lastly, because the proposed methods only alters the FDR estimation in the SAMseq procedure, the proposed methods can also be used to modify the original SAM method that uses different methods for calculating test statistics.

CHAPTER 4. COMPARISION OF PROPOSED METHOD II AND OTHER COMMONLY-USED EXISTING METHODS

4.1. Summary

In this chapter, the performance of proposed method II, the best-performing method from Chapter 3, to other commonly-used existing methods for identifying DE genes from RNA-Seq experiments are compared. These methods are NBPSeq (Yanming et al., 2011), edgeR (Robinson et al., 2010), and DESeq2(Love et al., 2014). Proposed method II is a non-parametric procedure described in section 3.3.2.2., while the NBPSeq, edgeR and DESeq2 are parametric methods that assume a negative binomial distribution for the data. NBPSeq, edgeR and DESeq2 first estimate the dispersion parameter and test statistics. The test statistics are then transformed into p-values and FDRs are estimated. DESeq2 and edgeR uses the Benjamini and Hochberg (1995) procedure to estimate the FDR for each gene, while the NBPSeq uses Storey's 2002 q-value approach.

4.2. Overview of DESeq2 NBPSep and edgeR methods

4.2.1. DESeq2 method

DESeq2 is a successor of DESeq, which was proposed by Anders and Huber (2010). In their previous method, they proposed using a negative binomial distribution with variance and mean linked by local regression to estimate the data variability and a suitable error model. To improve on the stability and interpretability of estimates, Love et al. (2014) proposed using shrinkage estimation for dispersions and fold changes which allows for more quantitative analysis (such as experiments with small number of replicates) based on the strength rather than the presence of differential expression.

4.2.2. NBPSeq method

NBPSeq method was developed by Yanming et al., 2011 a statistical method used to assess differential gene expression using RNA-Seq data. Yanming et al., (2011) propose the use of NBP parameterization of the negative binomial distribution to test for DE genes. Their method extends the exact test proposed by Robinson and Smyth (2007, 2008) by adding an extra parameter to allow the dispersion parameter to depend on the mean. Robinson and Smyth (2007, 2008) used a constant as a measure for the dispersion parameter, to model the count variability between biological replicates. To test for differentially expressed genes, log fold changes are estimated for each gene and the q-value method proposed by Storey (2002) is used to adjust the p-values control the false discovery rate.

4.2.3. edgeR method

EdgeR method was developed by Robinson et al., (2010) to examine differential expression of replicated count data using over dispersed Poisson model to account for both biological and technical variability. Robinson et al., (2010) uses the empirical Bayes procedures to shrink the dispersions towards a suitable value to measure the degree of over dispersion across transcripts, thereby improving the number of genes that are identified as differentially expressed. Lastly, to test for differentially expressed genes, likelihood-ratio statistics are estimated to compare the null hypothesis that a gene is equivalently expressed against a two-sided alternative that the gene is not equivalently expressed. The BH method proposed by Benjamini and Hochberg (1995) is then used to adjust the p-values control the false discovery rate. Robinson et al., (2010) method assumes data can be summarized into a table of counts, with rows corresponding to genes and columns to experimental units. The data is modeled as a negative binomial (NB) distribution.

4.3. Simulation studies

To evaluate the performance of proposed method II compared to the three commonly-used existing methods for estimating false discovery rate, data sets with Poisson distributed gene counts were randomly generated. For each data set, gene counts were randomly generated for m = 10,000 genes in two experiments. For gene j in experiment i, the gene count was generated using the procedures discussed in section 3.4.

Using the same simulation settings described in section 3.4, four different sample sizes, $n = \{4, 6, 10, 12\}$ and four different values for the number of EE genes, $m_0 = \{5000, 7000, 9000, 9500\}$ are used for the simulated data sets. To simulate asymmetry, five set of values representing the proportion of DE genes that are upregulated and downregulated were used: $\pi_1 = (0.5, 0.5)$, $\pi_2 = (0.7, 0.3)$, $\pi_3 = (0.8, 0.2)$, $\pi_3 = (0.9, 0.1)$, and $\pi_5 = (0.95, 0.05)$. For instance, in settings where $\pi_2 = (0.7, 0.3)$ is used, 0.7 represent the proportion of DE genes that are upregulated and 0.3 represent the proportion of DE genes that are downregulated in the data set. This results in eighty different simulation settings.

4.3.1. Results

For each simulation setting, 100 data sets were randomly generated. For each data set, all four methods (proposed method II, NBPSeq, edgeR, and DESeq2) were used to estimate the FDR for each gene to identify DE genes.

Controlling FDR at the 5% significance level, S (the number of DE genes DDE) for each data set was determined. To determine if each method controlled FDR at 5% significance level, the observed FDR, V/R (proportion of EE genes among all DDE genes) was calculated for each data set. If no genes were DDE for a particular data set, V/R was set to zero. For each simulation setting, paired *t*-tests were performed to test the difference in the mean *S* of proposed method II and NBPSeq, proposed method II and edgeR, and proposed method II and DESeq2 method. If a test between proposed method II and another existing method (NBPSeq, edgeR, or DESeq2) was significant at a type I error rate of 5% with the existing method outperforming proposed method, the higher mean *S* is underlined. If proposed method II outperformed all three other existing methods, the mean *S* for proposed method II is bolded. Table 5 and Table 6 below presents the mean S and mean V/R for each simulation setting, respectively. The corresponding standard errors for the mean S and mean V/R are reported in parentheses.

As expected, the power to detect DE genes increased as the number of EE genes decreased, that is, the number of DE genes (m_0) increased. Also, the power to identify DE genes increased as the sample size increased.

Proposed method II performed better than NBPSeq in 57 of 80 settings in terms of mean *S* (all settings with $n = 10$ and 12, and 17 of 20 settings with $n = 6$). Furthermore, proposed method II performed better than edgeR in 27 of 80 settings in terms of mean *S* (8 of 20 settings with $n = 6$, 10 of 20 settings with $n = 10$, and 9 of 20 settings with $n = 12$). Lastly, proposed method II performed better than DESeq2 in 52 of 80 settings in terms of mean *S* (15 of 20 settings with $n = 6$, 19 of 20 settings with $n = 10$, and 18 of 20 settings with $n = 12$). Proposed method II was outperformed by the NBPSeq, edgeR, and DESeq2 methods in all simulation settings with $n = 4$.

Again, looking at Table 6, NBPSeq, edgeR, and DESeq2 methods best controlled the observed FDR in settings where 50% (π_1) or 70% (π_2) of genes are upregulated or in settings where the number of EE genes is high ($m_0 = 9000$ or 9500). However, in settings where the level of asymmetry is high (π_3, π_4, and π_5) and the number of EE genes is smaller ($m_0 = 5000$ or 7000), the observed FDRs of these methods tend to be elevated above 5%, in many cases over 20%. In these simulation settings, the observed FDR for proposed method II exhibit much better control of the observed FDR, except for simulation settings with *n* = 4 as already noted.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting.

				Mean S			
n	***m₀***	**DE**	$\boldsymbol{\pi_i}$	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
4	**5000**	**5000**	$\boldsymbol{\pi_1}$	665.040 (4.746)	2393.740 (1.630)	3004.790 (1.870)	2361.140 (10.400)
			$\boldsymbol{\pi_2}$	671.570 (7.292)	2506.910 (1.204)	2751.190 (2.134)	2035.960 (8.545)
			$\boldsymbol{\pi_3}$	613.570 (3.819)	2225.980 (1.474)	2576.720 (1.726)	1948.730 (9.151)
			$\boldsymbol{\pi_4}$	802.560 (4.063)	2206.140 (1.388)	2512.640 (2.437)	1763.320 (5.946)
			$\boldsymbol{\pi_5}$	792.640 (4.38)	2033.790 (1.594)	2597.510 (3.802)	1891.770 (1.875)
	7000	**3000**	$\boldsymbol{\pi_1}$	120.560 (6.076)	1297.790 (1.019)	1432.570 (1.312)	1159.780 (3.647)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m₀***	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
4	**7000**	**3000**	π_2	208.450 (2.867)	1420.370 (1.186)	1519.580 (1.374)	1169.160 (1.472)
			π_3	250.930 (3.117)	1634.860 (1.084)	1852.370 (1.316)	1464.750 (3.526)
			π_4	300.450 (2.617)	1544.470 (1.091)	1741.700 (1.431)	1369.650 (4.226)
			π_5	324.180 (3.857)	1405.500 (1.080)	1748.680 (1.601)	1327.950 (5.149)
	9000	**1000**	π_1	<0.001 (<0.001)	462.250 (0.531)	510.120 (0.839)	372.800 (0.809)
			π_2	0.570 (0.412)	378.550 (0.517)	479.290 (0.805)	345.040 (1.038)
			π_3	4.650 (1.072)	595.450 (0.436)	614.120 (0.632)	504.430 (0.776)
			π_4	23.180 (2.111)	496.870 (0.671)	615.180 (0.627)	509.780 (2.021)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m0***	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
4	**9000**	**1000**	π_5	31.660 (2.404)	385.340 (0.627)	492.820 (0.784)	409.620 (2.510)
	9500	**500**	π_1	<0.001 (<0.001)	219.490 (0.394)	253.270 (0.580)	189.050 (0.558)
			π_2	<0.001 (<0.001)	196.620 (0.425)	264.890 (0.456)	208.370 (0.630)
			π_3	<0.001 (<0.001)	204.530 (0.420)	281.650 (0.427)	224.250 (1.236)
			π_4	0.330 (0.237)	217.160 (0.408)	267.780 (0.452)	220.880 (0.607)
			π_5	<0.001 (<0.001)	211.490 (0.392)	252.850 (0.455)	199.710 (0.676)
6	**5000**	**5000**	π_1	**3167.580** **(2.541)**	2512.510 (1.788)	3141.370 (1.935)	2881.370 (7.140)
			π_2	**3292.600** **(2.541)**	2436.120 (1.539)	2924.670 (1.696)	2829.200 (4.918)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m₀***	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
6	**5000**	**5000**	π_3	**3210.310** **(2.884)**	2427.720 (1.684)	3075.600 (2.298)	2655.170 (1.580)
			π_4	**3458.040** **(2.710)**	2943.060 (1.282)	3079.110 (1.751)	2693.820 (4.947)
			π_5	**3439.340** **(4.547)**	2728.340 (1.419)	2992.840 (2.130)	2598.970 (2.283)
	7000	**3000**	π_1	**1540.420** **(2.121)**	1329.570 (1.068)	1608.270 (1.484)	1398.550 (3.817)
			π_2	**1860.730** **(1.875)**	1473.040 (1.358)	1893.590 (1.519)	1672.560 (4.608)
			π_3	**1987.230** **(1.716)**	1679.790 (1.306)	1948.980 (1.400)	1733.900 (3.426)
			π_4	**1830.980** **(4.729)**	1364.390 (1.179)	1710.980 (1.560)	1477.740 (4.602)
			π_5	**1976.980** **(2.299)**	1581.580 (1.124)	1852.280 (1.593)	1570.240 (2.857)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m_0***	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
6	**9000**	**1000**	π_1	**535.970** **(5.080)**	452.860 (0.650)	559.940 (0.837)	507.750 (1.179)
			π_2	**517.720** **(6.150)**	475.560 (0.565)	588.800 (0.784)	513.550 (1.684)
			π_3	**578.540** **(3.576)**	512.040 (0.665)	618.890 (0.633)	553.620 (0.955)
			π_4	**542.750** **(4.241)**	498.480 (0.611)	580.510 (0.808)	521.350 (1.414)
			π_5	**491.180** **(1.252)**	463.450 (0.392)	490.860 (0.847)	416.830 (0.696)
	9500	**500**	π_1	**231.910** **(6.203)**	241.410 (0.436)	301.270 (0.526)	264.240 (0.757)
			π_2	**218.040** **(8.390)**	264.810 (0.378)	313.960 (0.578)	279.300 (0.695)
			π_3	**212.680** **(7.584)**	228.270 (0.440)	280.430 (0.532)	258.580 (0.948)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m₀***	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
6	**9500**	**500**	π_4	**259.150** **(3.650)**	251.460 (0.401)	281.560 (0.509)	260.610 (0.837)
			π_5	**262.940** **(3.350)**	220.760 (0.450)	275.860 (0.466)	244.630 (0.757)
10	**5000**	**5000**	π_1	**3338.570** **(2.454)**	2737.820 (1.628)	3307.910 (2.043)	3135.040 (4.275)
			π_2	**3555.450** **(2.499)**	2689.210 (1.413)	3417.290 (1.857)	3200.460 (1.735)
			π_3	**3668.730** **(2.497)**	2701.920 (1.725)	3474.850 (1.541)	3270.890 (1.721)
			π_4	**3621.910** **(2.080)**	2673.960 (1.788)	3199.810 (2.144)	3003.920 (1.816)
			π_5	**3831.440** **(2.445)**	2862.120 (1.533)	3367.310 (1.723)	3170.710 (1.636)
	7000	**3000**	π_1	**2049.050** **(1.542)**	1668.250 (1.178)	2091.140 (1.252)	1989.180 (2.639)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m*₀**	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
10	**7000**	**3000**	π_2	**1944.820** **(1.722)**	1610.330 (1.142)	1911.210 (1.458)	1811.090 (1.983)
			π_3	**2000.900** **(1.406)**	1646.71 (1.120)	1925.240 (1.482)	1791.640 (1.653)
			π_4	**2276.420** **(1.377)**	1907.520 (1.109)	2157.350 (1.263)	2025.280 (1.168)
			π_5	**2269.640** **(1.555)**	1789.000 (1.184)	2107.220 (1.621)	1972.130 (1.100)
	9000	**1000**	π_1	624.620 (0.762)	558.040 (0.588)	641.090 (0.709)	605.600 (1.046)
			π_2	**608.800** **(1.020)**	528.770 (0.641)	628.570 (0.796)	598.800 (1.209)
			π_3	**662.280** **(0.768)**	546.760 (0.659)	675.060 (0.685)	639.120 (1.019)
			π_4	**621.650** **(0.902)**	510.070 (0.631)	649.470 (0.774)	615.570 (0.884)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m₀***	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
10	**9000**	**1000**	π_5	**707.460** **(0.690)**	619.170 (0.520)	693.660 (0.636)	656.990 (0.906)
	9500	**500**	π_1	293.430 (0.555)	232.420 (0.443)	307.650 (0.559)	294.730 (0.672)
			π_2	**289.400** **(0.518)**	250.130 (0.333)	299.640 (0.464)	281.490 (0.571)
			π_3	**309.700** **(0.553)**	256.420 (0.425)	324.840 (0.486)	306.510 (0.624)
			π_4	**319.260** **(0.582)**	265.580 (0.360)	323.050 (0.516)	303.960 (0.682)
			π_5	**326.800** **(0.534)**	269.590 (0.458)	336.720 (0.439)	325.330 (0.559)
12	**5000**	**5000**	π_1	**3631.800** **(2.681)**	3044.920 (1.488)	3642.540 (1.530)	3503.160 (3.213)
			π_2	**3484.980** **(3.192)**	2852.450 (1.572)	3388.700 (1.649)	3232.940 (1.829)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m₀***	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
12	**5000**	**5000**	π_3	**3550.160** **(3.049)**	2819.240 (1.581)	3288.460 (2.148)	3113.280 (1.787)
			π_4	**3699.720** **(2.628)**	2664.210 (1.547)	3231.800 (1.804)	3047.890 (1.883)
			π_5	**3776.480** **(2.807)**	2687.840 (1.711)	3403.550 (1.662)	3282.990 (1.609)
	7000	**3000**	π_1	**2026.860** **(1.689)**	1677.590 (1.287)	2085.390 (1.373)	2000.710 (2.131)
			π_2	**2113.840** **(1.458)**	1791.330 (1.130)	2075.040 (1.407)	1978.530 (1.978)
			π_3	**2150.520** **(1.482)**	1773.460 (1.177)	2117.230 (1.289)	2026.720 (1.266)
			π_4	**2254.140** **(1.674)**	1795.050 (1.098)	2148.180 (1.352)	2044.430 (1.184)
			π_5	**2289.900** **(1.632)**	1711.590 (1.273)	2124.090 (1.532)	2019.690 (1.126)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m₀***	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
12	**9000**	**1000**	$\boldsymbol{\pi_1}$	655.110 (0.780)	538.450 (0.693)	671.320 (0.665)	641.550 (0.818)
			$\boldsymbol{\pi_2}$	**684.700** **(0.732)**	542.900 (0.673)	706.380 (0.638)	678.890 (0.829)
			$\boldsymbol{\pi_3}$	**653.430** **(0.849)**	553.950 (0.624)	666.360 (0.781)	635.380 (0.926)
			$\boldsymbol{\pi_4}$	**735.200** **(0.609)**	601.220 (0.650)	738.550 (0.675)	709.430 (0.696)
			$\boldsymbol{\pi_5}$	**708.540** **(0.778)**	602.530 (0.596)	696.570 (0.702)	668.830 (0.805)
	9500	**500**	$\boldsymbol{\pi_1}$	**311.570** **(0.511)**	253.510 (0.391)	327.320 (0.459)	316.630 (0.498)
			$\boldsymbol{\pi_2}$	**326.610** **(0.422)**	266.430 (0.427)	336.970 (0.441)	326.560 (0.517)
			$\boldsymbol{\pi_3}$	**301.850** **(0.436)**	265.480 (0.397)	312.910 (0.506)	297.680 (0.602)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 5. The mean S for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean S			
n	***m*₀**	**DE**	$\boldsymbol{\pi_i}$	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
12	**9500**	**500**	$\boldsymbol{\pi_4}$	**325.420** **(0.493)**	276.110 (0.394)	331.330 (0.394)	319.910 (0.511)
			$\boldsymbol{\pi_5}$	**323.060** **(0.498)**	285.790 (0.357)	327.210 (0.440)	312.950 (0.539)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the existing methods have a significant higher mean *S* compared to the proposed method II, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 6. The mean V/R for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting.

				Mean V/R			
n	***m*₀**	**DE**	$\boldsymbol{\pi_i}$	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
4	**5000**	**5000**	$\boldsymbol{\pi_1}$	0.161 (0.002)	0.015 (<0.001)	0.017 (<0.001)	<0.001 (<0.001)
			$\boldsymbol{\pi_2}$	0.142 (0.002)	0.087 (<0.001)	0.071 (0.001)	0.026 (0.001)
			$\boldsymbol{\pi_3}$	0.117 (0.001)	0.123 (<0.001)	0.161 (0.001)	0.052 (0.001)
			$\boldsymbol{\pi_4}$	0.100 (0.001)	0.197 (<0.001)	0.233 (0.002)	0.094 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 6. The mean V/R for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R			
n	***m₀***	**DE**	$\boldsymbol{\pi_i}$	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
4	**5000**	**5000**	$\boldsymbol{\pi_5}$	0.101 (0.001)	0.232 (0.001)	0.307 (0.004)	0.140 (0.001)
	7000	**3000**	$\boldsymbol{\pi_1}$	0.154 (0.006)	0.022 (<0.001)	0.021 (<0.001)	0.001 (<0.001)
			$\boldsymbol{\pi_2}$	0.158 (0.003)	0.046 (<0.001)	0.051 (0.001)	0.009 (<0.001)
			$\boldsymbol{\pi_3}$	0.173 (0.003)	0.083 (<0.001)	0.072 (0.001)	0.017 (<0.001)
			$\boldsymbol{\pi_4}$	0.177 (0.003)	0.114 (0.001)	0.116 (0.002)	0.035 (0.001)
			$\boldsymbol{\pi_5}$	0.159 (0.003)	0.106 (0.001)	0.123 (0.003)	0.042 (0.001)
	9000	**1000**	$\boldsymbol{\pi_1}$	<0.001 (<0.001)	0.026 (0.001)	0.028 (0.001)	<0.001 (<0.001)
			$\boldsymbol{\pi_2}$	0.005 (0.004)	0.027 (0.001)	0.025 (0.001)	<0.001 (<0.001)
			$\boldsymbol{\pi_3}$	0.046 (0.011)	0.039 (0.001)	0.038 (0.001)	0.002 (<0.001)
			$\boldsymbol{\pi_4}$	0.178 (0.016)	0.038 (0.001)	0.036 (0.001)	0.004 (<0.001)
			$\boldsymbol{\pi_5}$	0.195 (0.015)	0.044 (0.001)	0.057 (0.001)	0.006 (0.001)
	9500	**500**	$\boldsymbol{\pi_1}$	<0.001 (<0.001)	0.021 (0.001)	0.028 (0.001)	<0.001 (<0.001)
			$\boldsymbol{\pi_2}$	<0.001 (<0.001)	0.021 (0.001)	0.028 (0.001)	<0.001 (<0.001)
			$\boldsymbol{\pi_3}$	<0.001 (<0.001)	0.023 (0.001)	0.028 (0.001)	<0.001 (<0.001)
			$\boldsymbol{\pi_4}$	0.010 (0.007)	0.029 (0.001)	0.026 (0.001)	<0.001 (<0.001)
			$\boldsymbol{\pi_5}$	<0.001 (<0.001)	0.026 (0.001)	0.037 (0.001)	<0.0001 <0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 6. The mean V/R for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R			
n	***m₀***	**DE**	$\boldsymbol{\pi_i}$	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
6	**5000**	**5000**	$\boldsymbol{\pi_1}$	0.047 (<0.001)	0.014 (<0.001)	0.017 (<0.001)	0.003 (<0.001)
			$\boldsymbol{\pi_2}$	0.069 (0.001)	0.061 (<0.001)	0.043 (0.001)	0.077 (0.001)
			$\boldsymbol{\pi_3}$	0.034 (<0.001)	0.162 (<0.001)	0.249 (0.004)	0.143 (0.001)
			$\boldsymbol{\pi_4}$	0.034 (<0.001)	0.302 (<0.001)	0.296 (0.001)	0.236 (0.001)
			$\boldsymbol{\pi_5}$	0.036 (<0.001)	0.333 (<0.001)	0.364 (0.001)	0.288 (0.001)
	7000	**3000**	$\boldsymbol{\pi_1}$	0.038 (0.001)	0.021 (<0.001)	0.022 (<0.001)	0.002 (<0.001)
			$\boldsymbol{\pi_2}$	0.044 (<0.001)	0.048 (0.001)	0.050 (0.001)	0.020 (<0.001)
			$\boldsymbol{\pi_3}$	0.043 (0.001)	0.096 (<0.001)	0.108 (0.002)	0.049 (0.001)
			$\boldsymbol{\pi_4}$	0.051 (0.001)	0.109 (0.001)	0.121 (0.002)	0.079 (0.002)
			$\boldsymbol{\pi_5}$	0.047 (0.001)	0.159 (0.001)	0.155 (0.001)	0.090 (0.001)
	9000	**1000**	$\boldsymbol{\pi_1}$	0.063 (0.001)	0.027 (0.001)	0.027 (0.001)	0.002 (<0.001)
			$\boldsymbol{\pi_2}$	0.040 (0.001)	0.030 (0.001)	0.031 (0.001)	0.002 (<0.001)
			$\boldsymbol{\pi_3}$	0.044 (0.001)	0.030 (0.001)	0.036 (0.001)	0.004 (<0.001)
			$\boldsymbol{\pi_4}$	0.038 (0.001)	0.046 (0.001)	0.053 (0.001)	0.012 (0.001)
			$\boldsymbol{\pi_5}$	0.044 (0.001)	0.051 (0.001)	0.038 (0.001)	0.012 (<0.001)
	9500	**500**	$\boldsymbol{\pi_1}$	0.051 (0.003)	0.027 (0.001)	0.032 (0.001)	0.001 (<0.001)
			$\boldsymbol{\pi_2}$	0.064 (0.004)	0.027 (0.001)	0.030 (0.001)	0.001 (<0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 6. The mean V/R for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R			
n	***m*₀**	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
6	**9500**	**500**	π_3	0.081 (0.005)	0.026 (0.001)	0.033 (0.001)	0.003 (<0.001)
			π_4	0.058 (0.003)	0.041 (0.001)	0.040 (0.001)	0.003 (<0.001)
			π_5	0.058 (0.002)	0.031 (0.001)	0.026 (0.001)	0.002 (<0.001)
10	**5000**	**5000**	π_1	0.047 (<0.001)	0.019 (<0.001)	0.018 (<0.001)	0.008 (<0.001)
			π_2	0.047 (<0.001)	0.099 (<0.001)	0.141 (0.002)	0.119 (0.001)
			π_3	0.048 (<0.001)	0.175 (<0.001)	0.255 (0.002)	0.231 (0.001)
			π_4	0.046 (<0.001)	0.295 (<0.001)	0.361 (0.002)	0.325 (<0.001)
			π_5	0.089 (0.001)	0.344 (<0.001)	0.387 (0.001)	0.384 (<0.001)
	7000	**3000**	π_1	0.049 (0.001)	0.028 (<0.001)	0.028 (<0.001)	0.008 (<0.001)
			π_2	0.048 (0.001)	0.066 (<0.001)	0.094 (0.001)	0.048 (0.001)
			π_3	0.048 (0.001)	0.116 (0.001)	0.141 (0.003)	0.087 (0.001)
			π_4	0.052 (0.001)	0.198 (<0.001)	0.209 (0.003)	0.186 (0.001)
			π_5	0.055 (0.001)	0.215 (0.001)	0.251 (0.004)	0.223 (0.001)
	9000	**1000**	π_1	0.044 (0.001)	0.035 (0.001)	0.029 (0.001)	0.006 (<0.001)
			π_2	0.048 (0.001)	0.037 (0.001)	0.058 (0.001)	0.010 (<0.001)
			π_3	0.047 (0.001)	0.041 (0.001)	0.052 (0.002)	0.022 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 6. The mean V/R for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R			
n	***m₀***	**DE**	π_i	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
10	**9000**	**1000**	π_4	0.041 (0.001)	0.051 (0.001)	0.099 (0.003)	0.027 (0.001)
			π_5	0.048 (0.001)	0.069 (0.001)	0.084 (0.002)	0.040 (0.001)
	9500	**500**	π_1	0.077 (0.002)	0.032 (0.001)	0.028 (0.001)	0.007 (0.001)
			π_2	0.059 (0.001)	0.029 (0.001)	0.029 ()0.001	0.006 (<0.001)
			π_3	0.043 (0.001)	0.033 (0.001)	0.038 (0.001)	0.008 (0.001)
			π_4	0.050 (0.001)	0.031 (0.001)	0.036 (0.001)	0.011 (0.001)
			π_5	0.040 (0.001)	0.036 (0.001)	0.058 (0.002)	0.014 (0.001)
12	**5000**	**5000**	π_1	0.035 (<0.001)	0.024 (<0.001)	0.027 (0.001)	0.012 (<0.001)
			π_2	0.042 (0.001)	0.130 (<0.001)	0.130 (0.002)	0.137 (0.001)
			π_3	0.040 (<0.001)	0.228 (<0.001)	0.281 (0.002)	0.255 (<0.001)
			π_4	0.053 (0.001)	0.289 (<0.001)	0.322 (0.001)	0.333 (<0.001)
			π_5	0.053 (0.001)	0.327 (<0.001)	0.412 (0.001)	0.394 (<0.001)
	7000	**3000**	π_1	0.046 (<0.001)	0.027 (<0.001)	0.033 (0.001)	0.011 (<0.001)
			π_2	0.053 (0.001)	0.080 (<0.001)	0.068 (0.001)	0.063 (0.001)
			π_3	0.045 (0.001)	0.121 (0.001)	0.194 (0.004)	0.132 (0.001)
			π_4	0.056 (<0.001)	0.171 (0.001)	0.220 (0.004)	0.206 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 6. The mean V/R for proposed method II, NBPSeq, edgeR and DESeq2 methods with associated standard errors in parentheses for each simulation setting (continued).

				Mean V/R			
n	***m₀***	**DE**	$\boldsymbol{\pi_i}$	**Proposed method II**	**NBPSeq**	**edgeR**	**DESeq2**
12	**7000**	**3000**	$\boldsymbol{\pi_5}$	0.066 (0.001)	0.196 (0.001)	0.243 (0.004)	0.241 (0.001)
	9000	**1000**	$\boldsymbol{\pi_1}$	0.049 (0.001)	0.030 (0.001)	0.032 (0.001)	0.007 (<0.001)
			$\boldsymbol{\pi_2}$	0.045 (0.001)	0.033 (0.001)	0.049 (0.001)	0.013 (0.001)
			$\boldsymbol{\pi_3}$	0.045 (0.001)	0.046 (0.001)	0.059 (0.001)	0.024 (0.001)
			$\boldsymbol{\pi_4}$	0.053 (0.001)	0.058 (0.001)	0.078 (0.002)	0.041 (0.001)
			$\boldsymbol{\pi_5}$	0.049 (0.001)	0.070 (0.001)	0.087 (0.001)	0.056 (0.001)
	9500	**500**	$\boldsymbol{\pi_1}$	0.060 (0.001)	0.031 (0.001)	0.033 (0.001)	0.007 (0.001)
			$\boldsymbol{\pi_2}$	0.053 (0.001)	0.032 (0.001)	0.044 (0.001)	0.007 (<0.001)
			$\boldsymbol{\pi_3}$	0.042 (0.001)	0.034 (0.001)	0.038 (0.001)	0.008 (0.001)
			$\boldsymbol{\pi_4}$	0.043 (0.001)	0.037 (0.001)	0.041 (0.001)	0.012 (0.001)
			$\boldsymbol{\pi_5}$	0.048 (0.001)	0.033 (0.001)	0.041 (0.001)	0.014 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

4.4. Real data analysis

In this section, RNA-Seq data from a real gene expression experiment described by Bottomly et al. (2011) is reanalyzed using proposed method II, NBPSeq, edgeR and DESeq2 methods. The description of the data was previously discussed in section 3.5. The data consist of two classes (B6 and D2); with a total of *n* = 21 samples, n_1 = 10 B6 samples and n_2 = 11 D2 samples. The data set contains 36,536 genes, the total number of genes *m* = 13,932 were analyzed after filtering to remove genes without any reads.

The number of genes declared to be DE using proposed method II, NBPSeq, edgeR and DESeq2 methods while controlling FDR at 5% are summarized in Figure 4 below.

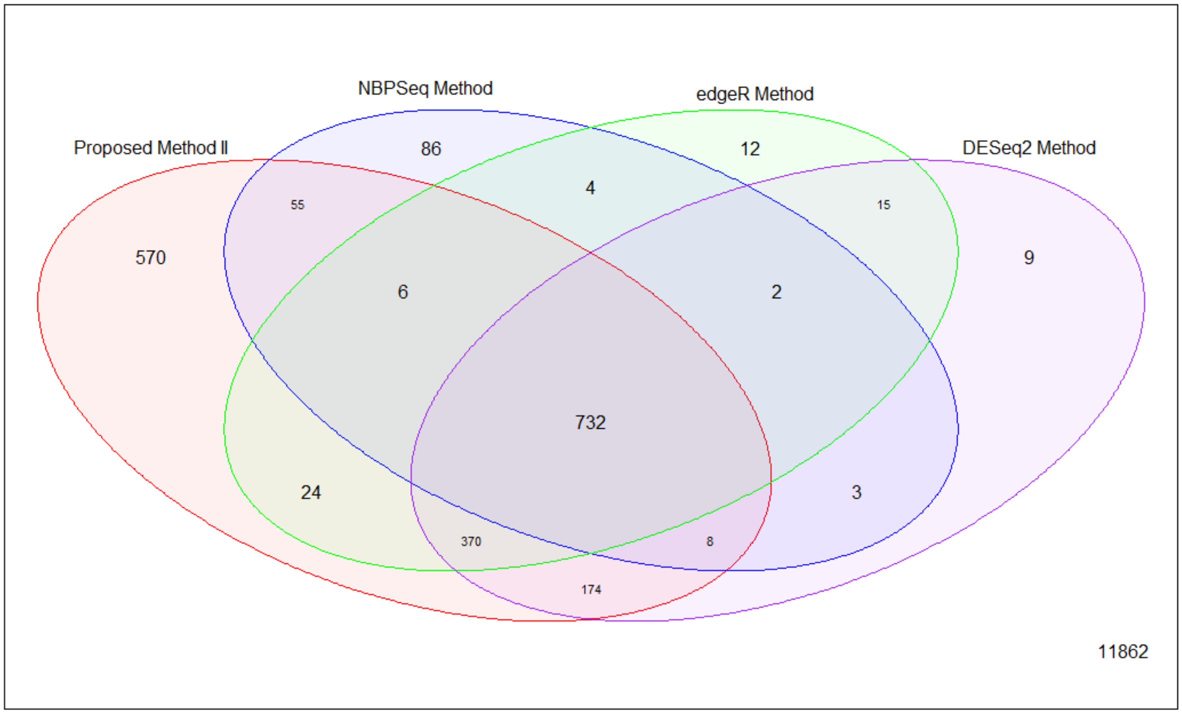

Figure 4. Venn diagram of genes declared to be DE for proposed method II, NBPSeq, edgeR and DESeq2 methods.

The total number of genes declared to be DE using all methods are summarized n Table 7 below. There were 732 genes that were DDE by all methods. An additional 570 genes were DDE by proposed method II. NBSeq method declared 86 more genes to be DE. 12 and 9 other genes were DDE using edgeR and DESeq2 method respectively. Hence, proposed method II declared the most genes to be DE, this is not surprising based on the results from the simulation studies in section 4.2. This analysis was performed on a real, not simulated, data set, therefore genes that are EE and DE are not known. Thus, evaluating the true FDR associated with each method cannot be done. However, because the sample size for each class is relatively large with a small degree of asymmetry, the estimation of the FDR is being adequately controlled at 5% based on the results of the simulation study in section 4.2.

Table 7. Total number of genes declared to be differentially expressed.

Method	**Total number of genes DDE**
Proposed method II	1939
DESeq2	1313
edgeR	1165
NBPSeq	896

4.5. Discussion

Proposed method II for estimating FDR, when there exists asymmetry in the distribution of the test statistics, has observed advantages over the commonly-used methods. Except for settings where $n = 4$, proposed method II generally outperformed NBPSeq, edgeR, and DESeq2 methods in terms of mean S in the settings where the number of EE genes was low ($m_0 = 5000$ and $m_0 = 7000$) and the degree of asymmetry was high (80%, 90%, and 95% of genes upregulated). The observed FDRs for NBPSeq, edgeR, and DESeq2 were also elevated in most of these setting. Therefore, using proposed method II when asymmetry in the test statistics is apparent and the estimated percentage of EE genes is low (less than 80%, for example) is recommended. When the estimated percentage of EE genes is high, use of the other methods is recommended. Using real RNA-Seq data, proposed method II declared more genes to be DE than the other methods at 5% significance level, which is consistent with the simulation results.

CHAPTER 5. MODIFICATION AND PERFORMANCE OF COMMONLY-USED PARAMETRIC METHODS WHEN THERE EXISTS ASYMMETRY IN THE DISTRIBUTION OF EFFECT SIZES IN IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES

5.1. Introduction

In chapters 3 and 4, the performance of SAMseq, its proposed modification, and three commonly-used methods were evaluated when there existed asymmetry in the distribution of the effect sizes in an RNA-Seq data set. In this chapter, performance of these three commonly-used parametric methods, DESeq2, NBPSeq and edgeR, when there exists asymmetry in the distribution of the effect sizes are evaluated. This research uses methods that modify the p-values of the commonly-used methods to account for asymmetry in the distribution of effect sizes when estimating false discovery rate (FDR). Additionally, through simulation studies and real data analysis, this research compares performance of these methods to that of the traditional BH proposed by Benjamini and Hochberg (1995), traditional q-value proposed by Storey (2002) and asymmetric q-value proposed by Orr et al. (2014). These methods were discussed in section 2.4.

5.2. Overview of DESeq2 method

DESeq2 is a successor of DESeq, which was proposed by Anders and Huber (2010). In their previous method, they proposed using a negative binomial distribution with variance and mean linked by local regression to estimate the data variability and a suitable error model. To improve the stability and interpretability of the estimates, Love et al. (2014) proposed using shrinkage estimation for dispersions and fold changes which allows for quantitative analysis (on experiments with small number of replicates, for example) based more on the strength rather than the presence of differential expression.

For the expression of gene *i* from experimental unit *j* $\left(G_{ij}\right)$ in each class, fit a generalized linear model using the negative binomial distribution with a logarithm link function, i.e.,

$$G_{ij} \sim NB\left(mean = \mu_{ij},\ dispersion = \alpha_i\right), \tag{5.1}$$

where the mean is estimated as

$$\mu_{ij} = s_{ij}\left(q_{ij}\right) \qquad i = 1, 2, ..., m, \qquad j = 1, 2, ...p\ .$$

(5.2)

s_{ij} is the normalization factor and considered constant within a sample, i.e., $s_{ij} = s_j$. s_j is estimated as

$$s_j = \underset{i=G_i^m \neq 0}{median}\left(\frac{G_{ij}}{G_i^m}\right) \tag{5.3}$$

and

$$G_i^m = \left(\prod_{j=1}^{p} G_{ij}\right)^{1/p}, \tag{5.4}$$

where *p* is the total number of units and G_i^p is the geometric mean estimate for each gene. The logarithm of q_{ij} is estimated as

$$\log_2 q_{ij} = \sum\nolimits_r x_{jr}\beta_{ir} \qquad r = 0, ...k-1\,. \tag{5.5}$$

x_{jr} is the design matrix element with coefficients β_{ir} and *r* is the covariate index with intercept *r* = 0 and *k* is the number of parameters. In a two class experiment, *j* indicates whether sample *j* is from the controlled samples or treated samples. The empirical Bayes shrinkage for dispersion estimation is modeled by the dispersion parameter α_i, which describes the variance of each gene as

$$Var(G_{ij}) = \mu_{ij} + \alpha_i \mu_{ij}^2, \tag{5.6}$$

α_i follows a log normal prior distribution that is centered around a trend, and depends on the gene's mean normalized read count. α_i is estimated as

$$\log \alpha_i \sim N\left(\log \alpha_{tr}\left(\bar{\mu}_i\right), \sigma_d^2\right), \tag{5.7}$$

where α_{tr} is a function of the gene's mean normalized read count. $\bar{\mu}_i$ describes the mean-dependent expectation of the prior and estimated as

$$\bar{\mu}_i = \frac{1}{p} \sum_j \frac{G_{ij}}{s_{ij}} \tag{5.8}$$

σ_d represents the width of the prior, which describes how much the individual genes' true dispersions scatter around the trend. The trend function is estimated as

$$\alpha_{tr}\left(\bar{\mu}\right) = \frac{\alpha_1}{\bar{\mu}} + \alpha_0 \tag{5.9}$$

where α_1 and α_0 are estimated by iteratively fitting a gamma-family GLM (Generalized Linear Model). To estimate the fold-change (FC) using the empirical Bayes procedure, Love *et al.*, (2014) outlined the following steps;

(1) Estimate the maximum-likelihood (MLE) for the logarithm of the FCs using ordinary GLM.

(2) Fit a zero-centered normal distribution to the observed distribution of the MLEs over all genes; thus assuming a normal prior for the coefficients β_{ir} (logarithm of the fold-changes) of the log link function

$$\beta_{ir} \sim N\left(0, \sigma_r^2\right) \tag{5.10}$$

Testing whether each model coefficients differ significantly from zero, the following procedures are used;

(1) Fit GLMs for each gene to obtain the shrunken logarithm of the FCs (LFCs) and estimate it corresponding standard errors.

(2) Estimate the test statistic (Wald test) with it corresponding p-values for each gene. The test statistic is estimated as

$$W_i = \frac{\hat{\beta}_{ir}}{se\left(\hat{\beta}_{ir}\right)}, \tag{5.11}$$

this result in a *z*-statistic which is then compared to a standard normal distribution.

(3) Estimate the filter statistic as the mean of the normalized counts for each gene.

(4) Remove genes with mean normalized counts less than a filtering threshold.

(5) Adjust for multiple hypothesis testing, the p-values corresponding to the subset of genes that passes the filtering procedure described in step 4 and 5, using the BH procedure.

5.2.1. Proposed modification for DESeq2 method p-values

To account for asymmetry in the distribution of the test statistic, this research proposes modifying the estimation of the adjusted p-values used to estimate the FDRs in DESeq2 method. The following steps outlines the proposed method for a two class experiment;

(1) Run the DESeq2 method to obtain the test statistic and the unadjusted p-values (raw p-values) that pass the filtering procedure for each gene.

(2) Divide the test statistics (W) into two groups based on the sign of the test statistics with their corresponding raw p-values. Thus, genes with positive test statistics $W^+ = W > 0$ and genes with negative test statistics $W^- = W \le 0$.

(3) Apply the BH method and asymmetric q-value method proposed by Orr et al. (2014) used to adjust the raw p-values for multiple hypothesis testing to each group separately.

These procedures will then be referred to as asymmetric BH method and asymmetric q-value method. All other procedures used in the DESeq2 method remain the same.

5.3. Overview of edgeR method

The edgeR method was developed by Robinson et al., (2010) to examine differential expression of replicated count data using an over dispersed Poisson model to account for both biological and technical variability. Robinson et al. (2010) uses an empirical Bayes procedure to shrink the dispersions towards a suitable value to measure the degree of over dispersion across transcripts, thereby improving the number of genes that are identified as differentially expressed. Lastly, to test for differentially expressed genes, likelihood-ratio statistics are estimated to compare the null hypothesis that a gene is equivalently expressed against a two-sided alternative that the gene is not equivalently expressed. The BH method proposed by Benjamini and Hochberg (1995) is then used to adjust the p-values to control the false discovery rate.

An assumption of the edgeR method assumes data can be modeled using a negative binomial (NB) distribution. For the expression of gene *i* from experimental unit *j* $\left(Y_{ij}\right)$ in each class,

$$Y_{ij} \sim NB\left(mean = M_j p_{ic},\ dispersion = \phi_i\right); \tag{5.12}$$

where M_j is the library size, i.e., the total number of reads from a specific experimental unit, ϕ_i is the dispersion parameter, and p_{ic} is the relative abundance of gene *i* in the class (*c*) in which the experimental unit *j* belongs.

5.3.1. Proposed method for edgeR method

A similar procedure proposed for modifying the FDR estimation for DESeq2 is employed here. Unlike DESeq2 which uses the Wald test to determine the test statistic, edgeR uses the log fold change. Likewise, to account for asymmetry in the distribution of the log fold change, this research proposes modifying the BH method used to estimate the FDRs in edgeR method. The following steps outline the proposed method for a two-class experiment;

(1) Run the edgeR method to obtain the log fold change and the p-value for each gene.

(2) Divide the log fold changes ($\log FC_{edgeR}$) into two groups based on the sign of the $\log FC_{edgeR}$ with their corresponding p-values. Thus, genes with positive $\log FC_{edgeR}$ $\log FC^{+}_{edgeR} = \log FC_{edgeR} > 0$ and genes with negative $\log FC_{edgeR}$ $\log FC^{-}_{edgeR} = \log FC_{edgeR} \leq 0$.

(3) Apply the BH method and asymmetric q-value proposed by Orr et al. (2014) used to adjust the p-values for multiple hypothesis testing to each group separately.

All other procedures used in the edgeR method remains the same.

5.4. Overview of NBPSeq method

The NBPSeq method, by Yanming et al. (2011), is a statistical method used to assess differential gene expression using RNA-Seq data. Yanming et al. (2011) proposes the use of NBP parameterization of the negative binomial distribution to test for DE genes. Their method extends the exact test proposed by Robinson and Smyth (2007, 2008) by adding an extra parameter to allow the dispersion parameter to depend on the mean. Robinson and Smyth (2007, 2008) used a constant as a measure for the dispersion parameter to model the count variability between biological replicates. To test for differentially expressed genes, log fold changes are estimated for each gene and the q-value method proposed by Storey (2002) is used to adjust the p-values to control the false discovery rate.

5.4.1. Proposed method for NBPSeq method

Similar to the procedures discussed in section 5.3.1 for modifying the estimation of the FDR, to account for asymmetry in the distribution of the log fold changes. This research proposes modifying the q-value method used to estimate the FDRs in NBPSeq method. The following steps outlines the proposed method for a two-class experiment.

(1) Run the NBPSeq method to obtain the log fold change and the p-value for each gene.

(2) Divide the log fold changes (logFC$_{NBPSeq}$) into two groups based on the sign of the logFC$_{NBPSeq}$ with their corresponding p-values. Thus, genes with positive logFC$_{NBPSeq}$ $\log FC^{+}_{NBPSeq} = \log FC_{NBPSeq} > 0$ and genes with negative logFC$_{NBPSeq}$ $\log FC^{-}_{NBPSeq} = \log FC_{NBPSeq} \leq 0$.

(3) Apply the asymmetric q-value method proposed by Orr et al. (2014) and BH method used to adjust the p-values for multiple hypothesis testing to each group separately.

All other procedures used in the NBPSeq method remains the same.

5.5. Simulation studies

Evaluating the performance of proposed BH and q-value methods compared to traditional BH method (Benjamini and Hochberg, 1995) and traditional q-value method (Storey, 2002) for estimating false discovery rate; data sets with Negative binomial distributed gene counts were randomly generated. For each data set, gene counts were randomly generated for m = 10,000 genes in two experiments. For gene i in experiment j, the gene count was generated as

$$G_{ij} \sim NB\left(\mu_{ij}, \phi_i\right) . \tag{5.13}$$

Using procedures implemented by Bi and Liu (2016), the mean μ_{ij} and the dispersion parameter ϕ_i was estimated based on a real RNA-Seq data set "Hammer" (Hammer, P. *et al.*, 2010). The experiment was performed to evaluate gene expression in the L4 dorsal root ganglion (DRG) of rats with chronic neuropathic pain induced by spinal nerve ligation (SNL) of the neighboring (L5) spinal nerve at two time points (2 weeks and 2 months after SNL). There were two classes (2 weeks and 2 months); with a total of 8 samples, 4 two weeks' samples and 4 two months' samples. A subset of the data consisting of samples after 2 weeks were used to estimate the mean and dispersion. The data set contains 29,516 genes, with many of the genes not having any reads. These genes were removed, and the remaining 18,463 were used. The raw data set is named after the first author of the paper and is available from ReCount project (Frazee et al., 2011) with an identifier "Hammer". The estimation of the fold change is assumed to follow a log-normal distribution;

$$fold_{change} \sim \log\!-normal\left(\log(2), 0.5\log(2)\right) \; . \tag{5.14}$$

To create differences in simulation settings, simulated data sets with four different sample sizes, $n = \{4, 6, 10, 12\}$ and four different values for the number of EE genes, $m_0 = \{5000, 7000, 9000, 9500\}$ were used. To simulate asymmetry, five set of values representing the proportion of DE genes that are upregulated and downregulated were used: $\pi_1 = (0.5, 0.5)$, $\pi_2 = (0.7, 0.3)$, $\pi_3 = (0.8, 0.2)$, $\pi_3 = (0.9, 0.1)$, and $\pi_5 = (0.95, 0.05)$. For instance, in settings where $\pi_3 = (0.9, 0.1)$ is used, 0.9 represent the proportion of DE genes that are upregulated and 0.1 represent the proportion of DE genes that are downregulated in the data set. This results in eighty different simulation settings.

5.5.1. Results

For each simulation setting, 100 data sets were randomly generated. For each data set, all four methods (traditional BH method, asymmetric BH method, traditional q-value method and asymmetric q-value method) were used to estimate the FDR for each gene to identify DE genes, using the DESeq2, edgeR and NBPSeq methods. Controlling FDR at the 5% significance level, *S* (the number of DE genes DDE) was determined for each data set. To determine if each method controlled FDR at 5% significance level, the observed FDR, V/R (proportion of EE genes among all DDE genes) was calculated for each data set. If no genes were DDE for a particular data set, V/R was set to zero.

Originally, Deseq2 and edgeR uses the traditional BH method to adjust p-values for multiple testing. For each simulation setting, paired *t*-tests were performed to test the difference in the mean *S* of the traditional BH and asymmetric BH methods, traditional BH and traditional q-values methods; traditional BH and asymmetric q-value methods, asymmetric BH and traditional q-value methods, asymmetric BH and traditional q-value methods, and traditional q-value and asymmetric q-value methods. If the test between these comparisons were significant at a type I error rate of 5%, then the higher mean *S* is shown in bold font. If a test between the asymmetric BH and traditional q-value methods was significant at a type I error rate of 5% with the traditional q-value method outperforming the asymmetric BH method, the higher mean *S* is underlined. Also, if a test between the asymmetric BH and asymmetric q-value methods was significant at a type I error rate of 5% with the asymmetric q-value method outperforming the asymmetric BH method, the higher mean *S* is *italicized*. Lastly, if a test between the traditional q-value method and asymmetric q-value methods was significant at a type I error rate of 5% with the asymmetric q-value method outperforming the traditional q-value method, the higher mean *S* is underlined.

On the other hand, NBPSeq uses the traditional q-value method to adjust p-values for multiple testing. Again, for each simulation setting, paired *t*-tests were performed to test the difference in the mean *S* of the traditional q-value and asymmetric q-value methods, traditional q-value and traditional BH methods, traditional q-value and asymmetric BH methods, asymmetric q-value and traditional BH methods, asymmetric q-value and asymmetric BH methods and, traditional BH and asymmetric BH methods. If the test between these comparisons were significant at a type I error rate of 5%, then the higher mean *S* is shown in bolded font. Like, the previous comparisons of the mean *S*, if a test between the asymmetric q-value and traditional BH methods was significant at a type I error rate of 5% with the traditional BH method outperforming the asymmetric q-value method, the higher mean *S* is underlined. Also, if a test between the asymmetric q-value and asymmetric BH methods was significant at a type I error rate of 5% with the asymmetric BH method outperforming the asymmetric q-value method, the higher mean *S* is *italicized*. Lastly, if a test between the traditional BH method and asymmetric BH methods was significant at a type I error rate of 5% with the asymmetric BH method outperforming the traditional BH method, the higher mean *S* is underlined.

Table 8 and Table 9 below presents the mean S and mean V/R for each simulation setting, respectively for DESeq2 method. Table 10 and Table 11 below presents the mean S and mean V/R for each simulation setting, respectively for NBPSeq. Table 12 and Table 13 below presents the mean S and mean V/R for each simulation setting, respectively for edgeR method. The corresponding standard errors for the mean S and mean V/R are reported in parentheses.

As expected, the power to detect DE genes increased as the number of EE genes decreased that is, the number of DE genes (m_0) increased. Also, the power to identify DE genes increased as the sample size increased.

The traditional BH method did not outperform the asymmetric BH, traditional q-value method and asymmetric q-value method in any of the simulation settings in terms of mean *S*, as seen in Table 8 (Deseq2 method). The asymmetric BH method performed better than the traditional BH method in 64 of the 80 simulation settings with regards to mean *S* (16 of 20 settings with n = 4, 6, 10, and 12). The traditional q-value method performed better than the traditional BH method in all the simulation settings. Also, the asymmetric q-value method performed better than the traditional BH method in 76 of the 80 simulations, including all settings with n = 6, 10, 12, and 16 of 20 settings with n = 4. Furthermore, the traditional q-value method performed better than the asymmetric BH method in 45 of 80 settings in terms of mean *S* (10 of 20 settings with n = 4, 11 of 20 settings with n = 6, 12 of 20 settings with n = 10, and 12 of 20 settings with n = 12). The asymmetric q-value method was outperformed by the asymmetric BH method in 17 of 20 simulation settings with n = 4 in terms of mean *S*. Comparing the performance of the traditional q-value method to the asymmetric q-value method, the asymmetric q-value method performed better than the traditional q-value method in 52 of the 80 settings in terms of the mean *S* (6 of 20 settings with n = 4, 16 of 20 settings with n = 6, 16 of 20 settings with n = 10, and 14 of 20 settings with n = 12).

Although a higher value of mean *S* was observed in most traditional BH method compared to the asymmetric BH method, in the 6 of 80 settings (n = 4, m0 = {7000, 9000, 9500}, and π_1, n =6, $m0$ = 9000, π_1, n =10, $m0$ = {7000, 9000} and π_1, and n =12, $m0$ = 5000 and π_1); these differences were not significant. Apart from these settings, a higher value of mean *S* was observed using the asymmetric BH method compared to traditional BH method in 7 of 80 settings, but there were no significant difference in mean *S* at 5%. Lastly, in settings where *n* = 6, *m0* = 7000, and π_1 or *n* = 12, *m0* = 9500 and π_1, the performance of traditional and asymmetric BH methods were the same in terms of the mean *S*.

As shown in Table 9, the observed FDR (mean V/R) was comparable among all the methods, with levels elevated above 5%. Apart from simulation settings with π_1, the asymmetric q-value method better controlled the observed FDR than the traditional BH method. In most settings, the asymmetric BH method compared to the traditional BH method better controlled the observed FDR close to or slightly higher than 5%.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting.

				DESeq2			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**5000**	**5000**	π_1	2174.280 (4.701)	2174.290 (4.688)	**2460.440** **(5.467)**	***2231.720*** ***(9.712)***
			π_2	1963.380 (4.823)	**1991.810** **(4.877)**	**2266.310** **(5.377)**	***2044.540*** ***(10.610)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**5000**	**5000**	π_3	1702.370 (4.431)	**1762.690** **(4.484)**	**2025.540** **(5.605)**	***1811.050*** ***(9.696)***
			π_4	1337.320 (3.968)	**1442.870** **(3.919)**	**1675.200** **(5.257)**	**1450.910** **(7.897)**
			π_5	1134.800 (4.161)	**1269.500** **(4.750)**	**1483.530** **(5.776)**	**1248.420** **(6.269)**
	7000	**3000**	π_1	1153.510 (3.221)	1153.490 (3.236)	**1230.790** **(3.707)**	1150.570 (3.804)
			π_2	1105.340 (3.745)	**1125.870** **(3.715)**	**1188.240** **(3.972)**	**1118.810** **(3.918)**
			π_3	1025.230 (3.841)	**1072.740** **(3.584)**	**1110.960** **(4.047)**	**1059.010** **(3.906)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**7000**	**1000**	π_4	905.500 (3.154)	**999.390** **(3.191)**	**998.770** **(3.739)**	**978.750** **(3.684)**
			π_5	827.950 (3.298)	**949.060** **(3.466)**	**924.000** **(3.913)**	**919.010** **(3.628)**
	9000	**1000**	π_1	298.720 (1.835)	298.110 (1.819)	**302.880** **(1.871)**	294.640 (1.824)
			π_2	293.990 (1.873)	**299.560** **(1.859)**	**298.330** **(1.947)**	**295.230** **(1.899)**
			π_3	285.050 (1.685)	**298.480** **(1.642)**	**289.720** **(1.749)**	**294.440** **(1.598)**
			π_4	278.090 (1.899)	**305.830** **(1.913)**	**283.170** **(1.972)**	**300.400** **(1.919)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m₀***	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**9000**	**1000**	$\boldsymbol{\pi_5}$	277.430 (1.810)	**314.380** **(1.803)**	**281.930** **(1.839)**	**308.460** **(1.819)**
	9500	**500**	$\boldsymbol{\pi_1}$	125.210 (1.215)	124.520 (1.238)	**125.990** **(1.237)**	122.460 (1.221)
			$\boldsymbol{\pi_2}$	130.050 (1.226)	**132.140** **(1.262)**	**130.730** **(1.218)**	129.690 (1.276)
			$\boldsymbol{\pi_3}$	129.470 (1.212)	**135.090** **(1.256)**	**130.240** **(1.209)**	**132.930** **(1.238)**
			$\boldsymbol{\pi_4}$	125.310 (1.301)	**138.330** **(1.386)**	**126.290** **(1.320)**	**135.150** **(1.349)**
			$\boldsymbol{\pi_5}$	124.990 (1.369)	**140.830** **(1.515)**	**125.840** **(1.401)**	**137.650** **(1.456)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**5000**	**5000**	π_1	2885.800 (4.269)	2885.930 (4.275)	**3153.090** **(4.737)**	***3151.710*** ***(4.775)***
			π_2	2650.820 (4.310)	**2687.500** **(4.274)**	**2964.230** **(5.197)**	***2987.110*** ***(5.242)***
			π_3	2344.490 (3.769)	**2419.520** **(3.730)**	**2726.330** **(4.601)**	***2774.560*** ***(4.642)***
			π_4	1940.650 (4.642)	**2056.530** **(4.942)**	**2397.020** **(6.083)**	***2468.140*** ***(7.291)***
			π_5	1710.940 (4.469)	**1852.350** **(4.191)**	**2208.920** **(5.867)**	***2271.590*** ***(10.943)***
	7000	**3000**	π_1	1600.600 (3.260)	1600.600 (3.251)	**1678.840** **(3.453)**	***1677.990*** ***(3.476)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**7000**	**3000**	π_2	1532.820 (3.346)	**1555.820** **(3.418)**	**1620.580** **(3.820)**	***1635.380*** ***(3.686)***
			π_3	1432.360 (2.947)	**1489.320** **(3.373)**	**1534.750** **(3.215)**	***1575.770*** ***(3.454)***
			π_4	1300.370 (3.201)	**1400.000** **(3.031)**	**1417.570** **(3.235)**	***1489.160*** ***(3.497)***
			π_5	1219.550 (3.364)	**1347.040** **(3.221)**	**1344.800** **(3.548)**	***1434.740*** ***(4.446)***
	9000	**1000**	π_1	447.260 (2.014)	447.240 (2.008)	**452.750** **(2.073)**	***452.230*** ***(2.034)***
			π_2	443.100 (1.975)	**448.810** **(1.957)**	**449.530** **(1.975)**	***453.630*** ***(2.001)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**9000**	**1000**	π_3	431.580 (1.822)	**446.800** **(1.893)**	**437.950** **(1.867)**	***450.500*** ***(1.883)***
			π_4	423.910 (1.913)	**451.180** **(1.893)**	**430.520** **(1.953)**	***452.740*** ***(1.900)***
			π_5	417.790 (1.890)	**454.880** **(1.832)**	**424.820** **(1.869)**	***456.490*** ***(1.848)***
	9500	**500**	π_1	201.370 (1.555)	201.400 (1.552)	**202.530** **(1.554)**	***202.170*** ***(1.537)***
			π_2	199.530 (1.281)	**202.310** **(1.319)**	**200.620** **(1.306)**	***202.910*** ***(1.332)***
			π_3	195.320 (1.233)	**202.070** **(1.316)**	**196.400** **(1.246)**	***202.740*** ***(1.324)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**9500**	**500**	π_4	195.650 (1.282)	**208.510** **(1.198)**	**196.730** **(1.293)**	***208.790*** ***(1.213)***
			π_5	194.780 (1.295)	**211.520** **(1.355)**	**196.180** **(1.301)**	**211.690** **(1.360)**
10	**5000**	**5000**	π_1	3560.800 (3.875)	3561.000 (3.880)	**3771.300** **(3.579)**	***3770.270*** ***(3.608)***
			π_2	3307.740 (3.983)	**3351.390** **(3.662)**	**3615.770** **(4.308)**	***3633.480*** ***(4.334)***
			π_3	3007.870 (3.254)	**3086.580** **(3.463)**	**3442.030** **(4.612)**	***3469.460*** ***(4.535)***
			π_4	2629.850 (3.273)	**2735.660** **(3.253)**	**3231.620** **(4.957)**	***3252.110*** ***(4.786)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m₀***	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**5000**	**5000**	$\boldsymbol{\pi_5}$	2404.710 (3.405)	**2514.990** **(3.547)**	**3107.660** **(4.968)**	***3117.580*** ***(4.686)***
	7000	**3000**	$\boldsymbol{\pi_1}$	2030.620 (2.772)	2030.570 (2.777)	**2096.580** **(2.963)**	***2096.180*** ***(2.963)***
			$\boldsymbol{\pi_2}$	1960.560 (3.350)	**1985.190** **(3.352)**	**2039.830** **(3.401)**	***2053.590*** ***(3.428)***
			$\boldsymbol{\pi_3}$	1866.050 (2.881)	**1921.470** **(2.790)**	**1967.220** **(2.911)**	***1999.110*** ***(2.859)***
			$\boldsymbol{\pi_4}$	1734.670 (2.774)	**1828.880** **(2.950)**	**1869.860** **(3.000)**	***1922.200*** ***(2.968)***
			$\boldsymbol{\pi_5}$	1653.050 (3.162)	**1771.060** **(3.084)**	**1810.730** **(3.410)**	***1875.450*** ***(3.350)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**9000**	**1000**	π_1	608.590 (1.873)	608.480 (1.858)	**614.240** **(1.870)**	***614.200*** ***(1.866)***
			π_2	599.490 (1.884)	**605.140** **(1.842)**	**605.560** **(1.907)**	***610.630*** ***(1.878)***
			π_3	594.930 (1.686)	**608.220** **(1.720)**	**601.400** **(1.704)**	***611.490*** ***(1.769)***
			π_4	580.640 (1.932)	**604.640** **(1.804)**	**587.880** **(1.914)**	***607.020*** ***(1.848)***
			π_5	571.550 (1.712)	**604.710** **(1.656)**	**579.720** **(1.722)**	***606.620*** ***(1.634)***
	9500	**500**	π_1	283.630 (1.118)	283.780 (1.124)	**285.120** **(1.125)**	***285.190*** ***(1.125)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**9500**	**500**	π_2	282.610 (1.255)	**285.170** **(1.278)**	**284.050** **(1.233)**	***285.970*** ***(1.260)***
			π_3	279.240 (1.183)	**285.940** **(1.172)**	**280.500** **(1.173)**	***286.440*** ***(1.162)***
			π_4	276.210 (1.178)	**287.770** **(1.213)**	**277.650** **(1.178)**	***288.050*** ***(1.198)***
			π_5	275.670 (1.104)	**290.140** **(1.140)**	**277.090** **(1.113)**	**290.250** **(1.140)**
12	**5000**	**5000**	π_1	3746.020 (3.372)	3745.970 (3.355)	**3934.540** **(3.237)**	***3933.660*** ***(3.247)***
			π_2	3506.880 (3.172)	**3549.850** **(3.148)**	**3803.040** **(3.379)**	***3818.430*** ***(3.357)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m₀***	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**5000**	**5000**	$\boldsymbol{\pi_3}$	3220.380 (3.202)	**3297.980** **(3.164)**	**3667.890** **(4.402)**	***3684.800*** ***(4.342)***
			$\boldsymbol{\pi_4}$	2848.750 (3.607)	**2942.000** **(3.594)**	**3507.860** **(5.665)**	***3508.100*** ***(5.403)***
			$\boldsymbol{\pi_5}$	2642.070 (3.460)	**2736.700** **(3.362)**	**3406.540** **(5.623)**	***3395.900*** ***(5.366)***
	7000	**3000**	$\boldsymbol{\pi_1}$	2153.170 (2.158)	2153.300 (2.510)	**2213.550** **(2.608)**	***2213.100*** ***(2.612)***
			$\boldsymbol{\pi_2}$	2085.230 (2.464)	**2109.620** **(2.421)**	**2160.510** **(2.763)**	***2172.230*** ***(2.696)***
			$\boldsymbol{\pi_3}$	2000.450 (2.747)	**2053.010** **(2.668)**	**2100.480** **(2.996)**	***2127.340*** ***(2.783)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**7000**	**3000**	π_4	1869.150 (3.074)	**1961.240** **(2.844)**	**2011.400** **(3.229)**	***2054.430*** ***(3.128)***
			π_5	1794.300 (2.791)	**1906.540** **(2.502)**	**1964.480** **(2.743)**	***2014.150*** ***(2.829)***
	9000	**1000**	π_1	656.220 (1.483)	656.370 (1.492)	**661.720** **(1.428)**	***661.540*** ***(1.421)***
			π_2	648.890 (1.622)	**654.430** **(1.689)**	**655.020** **(1.615)**	***658.850*** ***(1.676)***
			π_3	641.230 (1.816)	**654.110** **(1.730)**	**647.930** **(1.784)**	***657.700*** ***(1.770)***
			π_4	628.450 (1.678)	**652.520** **(1.643)**	**635.600** **(1.715)**	***654.310*** ***(1.659)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 8. The mean S for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**9000**	**1000**	π_5	619.410 (1.639)	**651.370** **(1.648)**	**627.490** **(1.676)**	***652.460*** ***(1.667)***
	9500	**500**	π_1	309.710 (1.250)	309.710 (1.251)	**310.830** **(1.223)**	***310.820*** ***(1.229)***
			π_2	307.800 (1.188)	**310.090** **(1.268)**	**309.250** **(1.207)**	***311.050*** ***(1.279)***
			π_3	306.720 (1.160)	**311.700** **(1.213)**	**307.680** **(1.174)**	***312.000*** ***(1.225)***
			π_4	300.150 (1.298)	**310.350** **(1.287)**	**301.580** **(1.287)**	***310.670*** ***(1.301)***
			π_5	302.550 (1.274)	**316.250** **(1.300)**	**304.130** **(1.308)**	**316.110** **(1.302)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 9. The mean V/R for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting.

				DESeq2			
				Mean V/R			
n	m_0	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**5000**	**5000**	π_1	0.033 (<0.001)	0.033 (<0.001)	0.048 (<0.001)	0.035 (0.001)
			π_2	0.051 (0.001)	0.044 (<0.001)	0.073 (0.001)	0.049 (0.001)
			π_3	0.077 (0.001)	0.059 (0.001)	0.111 (0.001)	0.066 (0.001)
			π_4	0.122 (0.001)	0.077 (0.001)	0.172 (0.001)	0.084 (0.001)
			π_5	0.160 (0.001)	0.083 (0.001)	0.219 (0.001)	0.089 (0.001)
	7000	**3000**	π_1	0.053 (0.001)	0.053 (0.001)	0.063 (0.001)	0.053 (0.001)
			π_2	0.062 (0.001)	0.054 (0.001)	0.074 (0.001)	0.054 (0.001)
			π_3	0.073 (0.001)	0.052 (0.001)	0.089 (0.001)	0.053 (0.001)
			π_4	0.093 (0.001)	0.049 (0.001)	0.114 (0.001)	0.050 (0.001)
			π_5	0.104 (0.001)	0.040 (0.001)	0.127 (0.001)	0.040 (0.001)
	9000	**1000**	π_1	0.105 (0.002)	0.105 (0.002)	0.108 (0.002)	0.102 (0.002)
			π_2	0.107 (0.002)	0.098 (0.002)	0.110 (0.002)	0.095 (0.002)
			π_3	0.110 (0.002)	0.092 (0.002)	0.113 (0.002)	0.090 (0.002)
			π_4	0.112 (0.002)	0.083 (0.001)	0.116 (0.002)	0.081 (0.001)
			π_5	0.118 (0.002)	0.078 (0.002)	0.121 (0.002)	0.076 (0.002)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 9. The mean V/R for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean V/R			
n	***m*₀**	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**9500**	**500**	$\boldsymbol{\pi_1}$	0.154 (0.002)	0.154 (0.003)	0.156 (0.002)	0.150 (0.003)
			$\boldsymbol{\pi_2}$	0.152 (0.003)	0.143 (0.003)	0.153 (0.003)	0.140 (0.003)
			$\boldsymbol{\pi_3}$	0.151 (0.003)	0.135 (0.003)	0.152 (0.003)	0.132 (0.003)
			$\boldsymbol{\pi_4}$	0.153 (0.003)	0.125 (0.003)	0.154 (0.003)	0.120 (0.003)
			$\boldsymbol{\pi_5}$	0.155 (0.003)	0.116 (0.003)	0.156 (0.003)	0.112 (0.003)
6	**5000**	**5000**	$\boldsymbol{\pi_1}$	0.034 (0.001)	0.034 (<0.001)	0.052 (0.001)	0.052 (<0.001)
			$\boldsymbol{\pi_2}$	0.060 (<0.001)	0.050 (<0.001)	0.092 (0.001)	0.081 (0.001)
			$\boldsymbol{\pi_3}$	0.100 (0.001)	0.076 (0.001)	0.153 (0.001)	0.128 (0.001)
			$\boldsymbol{\pi_4}$	0.166 (0.001)	0.110 (0.001)	0.241 (0.001)	0.193 (0.001)
			$\boldsymbol{\pi_5}$	0.211 (0.001)	0.129 (0.001)	0.269 (0.001)	0.231 (0.002)
	7000	**3000**	$\boldsymbol{\pi_1}$	0.054 (0.001)	0.054 (0.001)	0.066 (0.001)	0.066 (0.001)
			$\boldsymbol{\pi_2}$	0.066 (0.001)	0.055 (0.001)	0.082 (0.001)	0.071 (0.001)
			$\boldsymbol{\pi_3}$	0.081 (0.001)	0.055 (0.001)	0.103 (0.001)	0.075 (0.001)
			$\boldsymbol{\pi_4}$	0.108 (0.001)	0.054 (0.001)	0.139 (0.001)	0.078 (0.001)
			$\boldsymbol{\pi_5}$	0.126 (0.001)	0.047 (0.001)	0.163 (0.001)	0.071 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 9. The mean V/R for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean V/R			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**9000**	**1000**	π_1	0.097 (0.001)	0.097 (0.001)	0.102 (0.001)	0.101 (0.001)
			π_2	0.100 (0.001)	0.091 (0.001)	0.104 (0.001)	0.096 (0.001)
			π_3	0.101 (0.001)	0.086 (0.001)	0.107 (0.001)	0.089 (0.001)
			π_4	0.106 (0.002)	0.073 (0.001)	0.111 (0.002)	0.076 (0.001)
			π_5	0.110 (0.001)	0.069 (0.001)	0.116 (0.002)	0.072 (0.001)
	9500	**500**	π_1	0.127 (0.002)	0.127 (0.002)	0.129 (0.002)	0.129 (0.002)
			π_2	0.133 (0.003)	0.126 (0.002)	0.135 (0.003)	0.128 (0.002)
			π_3	0.130 (0.003)	0.118 (0.002)	0.133 (0.003)	0.120 (0.002)
			π_4	0.136 (0.002)	0.110 (0.002)	0.139 (0.002)	0.111 (0.002)
			π_5	0.135 (0.002)	0.102 (0.002)	0.138 (0.002)	0.102 (0.002)
10	**5000**	**5000**	π_1	0.033 (<0.001)	0.033 (<0.001)	0.054 (<0.001)	0.054 (<0.001)
			π_2	0.077 (<0.001)	0.063 (<0.001)	0.126 (0.001)	0.114 (0.001)
			π_3	0.138 (0.001)	0.105 (0.001)	0.244 (0.001)	0.203 (0.001)
			π_4	0.237 (0.001)	0.181 (0.001)	0.348 (0.001)	0.333 (0.001)
			π_5	0.269 (0.001)	0.230 (0.001)	0.407 (0.001)	0.399 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 9. The mean V/R for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean V/R			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**7000**	**3000**	π_1	0.050 (<0.001)	0.050 (<0.001)	0.065 (0.001)	0.064 (0.001)
			π_2	0.071 (0.001)	0.057 (0.001)	0.092 (0.001)	0.079 (0.001)
			π_3	0.097 (0.001)	0.064 (0.001)	0.131 (0.001)	0.096 (0.001)
			π_4	0.138 (0.001)	0.068 (0.001)	0.191 (0.001)	0.118 (0.001)
			π_5	0.167 (0.001)	0.066 (0.001)	0.233 (0.001)	0.131 (0.001)
	9000	**1000**	π_1	0.084 (0.001)	0.084 (0.001)	0.090 (0.001)	0.090 (0.001)
			π_2	0.088 (0.001)	0.082 (0.001)	0.094 (0.001)	0.087 (0.001)
			π_3	0.092 (0.001)	0.075 (0.001)	0.099 (0.001)	0.080 (0.001)
			π_4	0.099 (0.001)	0.066 (0.001)	0.106 (0.001)	0.071 (0.001)
			π_5	0.103 (0.001)	0.059 (0.001)	0.112 (0.001)	0.063 (0.001)
	9500	**500**	π_1	0.110 (0.002)	0.110 (0.002)	0.113 (0.002)	0.113 (0.002)
			π_2	0.109 (0.002)	0.104 (0.002)	0.112 (0.002)	0.106 (0.002)
			π_3	0.114 (0.002)	0.102 (0.002)	0.114 (0.002)	0.104 (0.002)
			π_4	0.117 (0.002)	0.092 (0.002)	0.121 (0.002)	0.093 (0.002)
			π_5	0.113 (0.002)	0.081 (0.002)	0.117 (0.002)	0.082 (0.002)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 9. The mean V/R for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean V/R			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**5000**	**5000**	π_1	0.032 (<0.001)	0.032 (<0.001)	0.054 (<0.001)	0.054 (<0.001)
			π_2	0.084 (<0.001)	0.068 (<0.001)	0.141 (0.001)	0.129 (0.001)
			π_3	0.160 (0.001)	0.124 (0.001)	0.259 (0.001)	0.244 (0.001)
			π_4	0.266 (0.001)	0.214 (0.001)	0.388 (0.001)	0.385 (0.001)
			π_5	0.332 (0.001)	0.278 (0.001)	0.441 (0.001)	0.444 (0.001)
	7000	**3000**	π_1	0.050 (0.001)	0.050 (0.001)	0.064 (0.001)	0.064 (0.001)
			π_2	0.072 (0.001)	0.057 (0.001)	0.096 (0.001)	0.081 (0.001)
			π_3	0.107 (0.001)	0.069 (0.001)	0.145 (0.001)	0.109 (0.001)
			π_4	0.155 (0.001)	0.078 (0.001)	0.220 (0.001)	0.145 (0.001)
			π_5	0.187 (0.001)	0.079 (0.001)	0.267 (0.001)	0.170 (0.001)
	9000	**1000**	π_1	0.081 (0.001)	0.081 (0.001)	0.086 (0.001)	0.086 (0.001)
			π_2	0.084 (0.001)	0.077 (0.001)	0.090 (0.001)	0.082 (0.001)
			π_3	0.090 (0.001)	0.072 (0.001)	0.098 (0.001)	0.078 (0.001)
			π_4	0.098 (0.001)	0.064 (0.001)	0.107 (0.001)	0.069 (0.001)
			π_5	0.102 (0.001)	0.054 (0.001)	0.111 (0.001)	0.058 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 9. The mean V/R for proposed FDR methods using DESeq2 with associated standard errors in parentheses for each simulation setting (continued).

				DESeq2			
				Mean V/R			
n	***m*₀**	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**9500**	**500**	$\boldsymbol{\pi_1}$	0.104 (0.002)	0.104 (0.002)	0.107 (0.002)	0.107 (0.002)
			$\boldsymbol{\pi_2}$	0.102 (0.002)	0.096 (0.002)	0.105 (0.002)	0.100 (0.002)
			$\boldsymbol{\pi_3}$	0.107 (0.002)	0.094 (0.002)	0.109 (0.002)	0.096 (0.002)
			$\boldsymbol{\pi_4}$	0.108 (0.002)	0.086 (0.002)	0.112 (0.002)	0.088 (0.002)
			$\boldsymbol{\pi_5}$	0.112 (0.002)	0.079 (0.001)	0.116 (0.002)	0.080 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

The traditional q-value method did not outperform the asymmetric q-value method in most of the simulation settings in terms of mean *S*, as seen in Table 10 (NBPSeq method). The asymmetric q-value method performed better than the traditional q-value method in 65 of the 80 simulation settings with regard to mean *S* (16 of 20 settings with *n* = 4, 6 and 10 and 17 of 20 settings with *n* = 12). The traditional q-value method performed better than the traditional BH method in all the simulation settings. Also, the asymmetric q-value method performed better than the traditional and asymmetric BH methods in all settings. Furthermore, the traditional q-value method performed better than the asymmetric BH method in 37 of 80 settings in terms of mean *S* (10 of 20 settings with *n* = 4 and 6, 8 of 20 settings with *n* = 10, and 9 of 20 settings with *n* = 10). Comparing the performance of the traditional BH method to the asymmetric BH method, the asymmetric BH method performed better than the traditional BH method in 65 of the 80 settings in terms of the mean *S* (16 of 20 settings with $n = 4$, 6 and 10, and 17 of 20 settings with $n = 12$).

Although a higher value of mean *S* was observed for the traditional q-value method compared to the traditional BH method in most simulation settings, these differences were not significant. There were no significant difference in mean *S* at 5% between all methods in 9 of 80 settings ($n = 4$, $m0 = \{5000, 9500\}$ and π_1, $n = 6$, $m0 = \{5000, 7000, 9500\}$ and π_1, $n = 10$, $m0 = \{7000, 9000\}$ and π_1, $n = 12$, $m0 = \{5000, 7000\}$ and π_1).

As shown in Table 11, the observed FDR (mean V/R) was similar among all the methods, with levels elevated above 5%. Apart from simulation settings with π_1, the asymmetric q-value method better controlled the observed FDR than the traditional q-value method. In most settings, the asymmetric BH method compared to the traditional q-value and asymmetric q-value methods better controlled the observed FDR close to or slightly higher than 5%.

Table 10. The mean S for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting.

				NBPSeq			
				Mean *S*			
n	***m*₀**	**DE**	**π_i**	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**5000**	**5000**	π_1	2091.990 (4.809)	2091.440 (4.812)	2350.050 (5.713)	2349.010 (5.650)
			π_2	1865.770 (5.120)	1898.470 (5.279)	2134.800 (5.520)	**2158.050** **(5.666)**
			π_3	1595.030 (4.731)	1662.220 (4.945)	1879.190 (5.671)	**1938.950** **(5.780)**
			π_4	1217.340 (4.309)	1331.980 (4.467)	1502.990 (5.821)	**1612.020** **(5.853)**
			π_5	1002.590 (3.902)	1153.300 (4.468)	1293.160 (5.914)	**1435.450** **(5.957)**
	7000	**3000**	π_1	1087.310 (3.459)	1086.880 (3.477)	1145.890 (3.932)	1145.420 (3.921)
			π_2	1037.050 (4.022)	1060.280 (4.049)	1101.070 (4.257)	**1118.920** **(4.138)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean S			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**7000**	**3000**	π_3	951.320 (3.959)	1005.000 (3.959)	1017.290 (4.113)	**1064.400** **(4.010)**
			π_4	828.880 (3.635)	**930.410** **(3.667)**	897.230 (3.932)	**988.090** **(4.013)**
			π_5	748.170 (3.482)	**883.590** **(3.544)**	819.990 (3.942)	**939.410** **(3.787)**
	9000	**1000**	π_1	267.780 (1.850)	267.920 (1.828)	268.290 (1.850)	268.560 (1.830)
			π_2	263.930 (2.041)	**269.520** **(1.885)**	264.410 (2.052)	**269.470** **(1.913)**
			π_3	256.280 (1.695)	**272.870** **(1.621)**	256.800 (1.726)	**272.070** **(1.659)**
			π_4	248.670 (1.885)	**280.940** **(1.895)**	249.300 (1.918)	**279.420** **(1.942)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean S			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**9000**	**1000**	π_5	247.630 (1.732)	**288.190** **(1.883)**	248.300 (1.745)	**286.120** **(1.908)**
	9500	**500**	π_1	109.030 (1.210)	108.780 (1.227)	109.030 (1.210)	108.870 (1.225)
			π_2	112.950 (1.309)	**116.500** **(1.334)**	112.950 (1.309)	**116.360** **(1.326)**
			π_3	113.130 (1.236)	**120.560** **(1.252)**	113.130 (1.236)	**120.060** **(1.249)**
			π_4	109.880 (1.378)	**123.960** **(1.459)**	109.880 (1.378)	**123.580** **(1.449)**
			π_5	108.190 (1.375)	**126.730** **(1.349)**	108.220 (1.349)	**125.780** **(1.339)**
6	**5000**	**5000**	π_1	2812.560 (4.355)	2812.520 (4.336)	3056.840 (4.949)	3055.910 (4.891)

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean S			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**5000**	**5000**	π_2	2565.210 (4.185)	2604.030 (4.095)	2853.280 (5.559)	**2880.140** **(5.560)**
			π_3	2248.480 (3.963)	2329.500 (4.240)	2600.520 (4.727)	**2657.860** **(4.757)**
			π_4	1831.860 (4.817)	1965.700 (5.063)	2251.000 (6.377)	**2344.580** **(6.153)**
			π_5	1593.450 (4.450)	1755.150 (4.274)	2042.880 (5.916)	**2155.190** **(5.805)**
	7000	**3000**	π_1	1544.050 (3.276)	1543.470 (3.301)	1601.510 (3.437)	1600.440 (3.438)
			π_2	1472.670 (3.705)	1497.330 (3.757)	1540.730 (3.999)	**1559.470** **(4.013)**
			π_3	1371.180 (3.210)	1432.510 (3.263)	1452.890 (3.370)	**1497.590** **(3.427)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean S			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**7000**	**3000**	π_4	1235.090 (3.181)	**1347.300** **(3.269)**	1333.260 (3.611)	**1414.650** **(3.397)**
			π_5	1151.390 (3.219)	**1294.590** **(3.113)**	1256.480 (3.374)	**1368.340** **(3.392)**
	9000	**1000**	π_1	417.710 (1.985)	417.740 (1.966)	418.370 (1.990)	418.350 (2.001)
			π_2	416.850 (2.031)	**423.640** **(1.992)**	418.000 (2.029)	**423.580** **(2.008)**
			π_3	406.570 (1.834)	**423.500** **(1.936)**	407.480 (1.858)	**422.210** **(1.944)**
			π_4	399.380 (1.930)	**430.830** **(1.952)**	400.570 (1.915)	**428.010** **(1.967)**
			π_5	394.720 (1.765)	**436.860** **(1.829)**	396.620 (1.777)	**433.910** **(1.835)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean S			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**9500**	**500**	π_1	185.870 (1.498)	185.900 (1.501)	185.870 (1.498)	185.890 (1.499)
			π_2	185.360 (1.367)	**188.140** **(1.373)**	185.390 (1.366)	**187.850** **(1.382)**
			π_3	181.500 (1.259)	**188.540** **(1.247)**	181.500 (1.259)	**188.090** **(1.257)**
			π_4	182.810 (1.227)	**195.910** **(1.214)**	182.870 (1.228)	**195.210** **(1.206)**
			π_5	182.470 (1.264)	**200.990** **(1.264)**	182.550 (1.260)	**199.840** **(1.278)**
10	**5000**	**5000**	π_1	3528.990 (3.918)	3528.740 (3.935)	3720.190 (3.667)	3720.320 (3.684)
			π_2	3268.830 (3.860)	3314.150 (3.827)	3552.550 (4.385)	**3575.570** **(4.247)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**5000**	**5000**	π_3	2957.440 (3.616)	3044.690 (3.645)	3363.750 (4.480)	**3402.370** **(4.364)**
			π_4	2556.780 (3.570)	2679.950 (3.392)	3130.980 (5.011)	**3170.250** **(4.912)**
			π_5	2319.730 (3.301)	2450.820 (3.534)	2991.400 (4.616)	**3021.540** **(4.361)**
	7000	**3000**	π_1	2003.400 (2.951)	2003.010 (2.983)	2055.020 (3.024)	2054.840 (3.002)
			π_2	1935.480 (3.334)	1961.800 (3.365)	2000.800 (3.400)	**2015.290** **(3.441)**
			π_3	1836.870 (2.704)	1895.580 (2.673)	1922.360 (2.759)	**1957.900** **(2.837)**
			π_4	1700.230 (2.877)	1801.760 (2.848)	1816.190 (2.909)	**1877.320** **(3.060)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**7000**	**3000**	π_5	1617.430 (3.198)	1746.590 (3.231)	1753.280 (3.596)	**1830.940** **(3.597)**
	9000	**1000**	π_1	594.700 (1.962)	594.550 (1.964)	596.130 (1.999)	595.940 (1.993)
			π_2	588.240 (1.699)	**593.450** **(1.740)**	589.810 (1.724)	**594.080** **(1.755)**
			π_3	583.540 (1.751)	**598.080** **(1.807)**	585.370 (1.803)	**596.910** **(1.829)**
			π_4	570.500 (1.975)	**597.700** **(1.976)**	572.910 (2.004)	**594.870** **(2.010)**
			π_5	562.880 (1.682)	**597.970** **(1.644)**	565.610 (1.689)	**594.940** **(1.668)**
	9500	**500**	π_1	275.510 (1.110)	275.510 (1.122)	275.540 (1.110)	275.600 (1.130)

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**9500**	**500**	π_2	275.400 (1.310)	**277.830** **(1.296)**	275.470 (1.311)	**277.650** **(1.290)**
			π_3	273.410 (1.207)	**279.680** **(1.146)**	273.430 (1.206)	**279.110** **(1.130)**
			π_4	272.210 (1.237)	**283.250** **(1.226)**	272.230 (1.237)	**281.870** **(1.212)**
			π_5	271.050 (1.193)	**286.800** **(1.183)**	271.090 (1.189)	**285.370** **(1.193)**
12	**5000**	**5000**	π_1	3724.160 (3.275)	3724.060 (3.321)	3894.170 (3.353)	3894.140 (3.356)
			π_2	3480.230 (3.374)	3526.930 (3.347)	3751.650 (3.691)	**3771.640** **(3.613)**
			π_3	3180.510 (3.268)	3264.240 (3.139)	3601.520 (4.377)	**3628.800** **(4.302)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**5000**	**5000**	π_4	2788.960 (3.466)	2897.990 (3.705)	3418.100 (5.737)	**3432.790** **(5.526)**
			π_5	2573.970 (3.471)	2685.680 (3.115)	3308.950 (5.685)	**3312.840** **(5.392)**
	7000	**3000**	π_1	2135.850 (2.533)	2135.860 (2.508)	2183.390 (2.663)	2183.390 (2.638)
			π_2	2068.930 (2.487)	2092.990 (2.569)	2130.220 (2.688)	**2143.780** **(2.699)**
			π_3	1982.130 (2.744)	2036.520 (2.539)	2066.420 (2.816)	**2097.200** **(2.780)**
			π_4	1845.480 (2.758)	1944.980 (2.946)	1970.920 (3.253)	**2023.190** **(3.132)**
			π_5	1766.510 (2.884)	1889.140 (2.666)	1916.740 (3.101)	**1978.880** **(2.899)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean *S*			
n	***m***	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**9000**	**1000**	$\boldsymbol{\pi_1}$	645.960 (1.516)	646.090 (1.518)	647.230 (1.493)	647.370 (1.485)
			$\boldsymbol{\pi_2}$	641.600 (1.756)	**646.880** **(1.757)**	643.470 (1.765)	**647.350** **(1.752)**
			$\boldsymbol{\pi_3}$	635.950 (1.706)	**648.330** **(1.637)**	637.660 (1.707)	**647.550** **(1.654)**
			$\boldsymbol{\pi_4}$	622.020 (1.699)	**648.680** **(1.853)**	624.680 (1.747)	**645.720** **(1.882)**
			$\boldsymbol{\pi_5}$	614.550 (1.743)	**647.140** **(1.750)**	617.680 (1.773)	**643.750** **(1.817)**
	9500	**500**	$\boldsymbol{\pi_1}$	303.170 (1.248)	**303.520** **(1.224)**	303.200 (1.249)	**303.580** **(1.220)**
			$\boldsymbol{\pi_2}$	303.790 (1.177)	**306.160** **(1.207)**	303.820 (1.178)	**305.800** **(1.218)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 10. The mean *S* for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean *S*			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**9500**	**500**	π_3	304.070 (1.231)	**309.150** **(1.324)**	304.100 (1.233)	**308.530** **(1.301)**
			π_4	297.830 (1.204)	**309.340** **(1.155)**	297.910 (1.207)	**308.070** **(1.145)**
			π_5	299.710 (1.325)	**314.280** **(1.355)**	299.780 (1.325)	**312.430** **(1.361)**

For each setting, the significant higher mean S value at 5% significance level is shown in bolded fonts. If the asymmetric BH method has a significant higher mean *S* compared to the traditional BH method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 11. The mean V/R for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting.

				NBPSeq			
				Mean V/R			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**5000**	**5000**	π_1	0.030 (<0.001)	0.030 (<0.001)	0.041 (<0.001)	0.041 (<0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 11. The mean V/R for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean V/R			
n	m_0	DE	π_i	Traditional BH	Asymmetric BH	Traditional QV	Asymmetric QV
4	**5000**	**5000**	π_2	0.044 (0.001)	0.038 (0.001)	0.061 (0.001)	0.054 (0.001)
			π_3	0.066 (0.001)	0.049 (0.001)	0.091 (0.001)	0.071 (0.001)
			π_4	0.102 (0.001)	0.060 (0.001)	0.141 (0.001)	0.090 (0.001)
			π_5	0.132 (0.001)	0.060 (0.001)	0.181 (0.001)	0.094 (0.001)
	7000	**3000**	π_1	0.048 (0.001)	0.048 (0.001)	0.055 (0.001)	0.055 (0.001)
			π_2	0.056 (0.001)	0.049 (0.001)	0.063 (0.001)	0.056 (0.001)
			π_3	0.063 (0.001)	0.046 (0.001)	0..073 (0.001)	0.053 (0.001)
			π_4	0.078 (0.001)	0.041 (0.001)	0.091 (0.001)	0.049 (0.001)
			π_5	0.086 (0.001)	0.033 (0.001)	0.102 (0.001)	0.040 (0.001)
	9000	**1000**	π_1	0.098 (0.002)	0.098 (0.002)	0.099 (0.002)	0.099 (0.002)
			π_2	0.099 (0.002)	0.092 (0.002)	0.100 (0.002)	0.092 (0.092)
			π_3	0.101 (0.002)	0.088 (0.002)	0.102 (0.002)	0.088 (0.002)
			π_4	0.105 (0.002)	0.079 (0.002)	0.105 (0.002)	0.079 (0.002)
			π_5	0.107 (0.002)	0.074 (0.002)	0.108 (0.002)	0.074 (0.002)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 11. The mean V/R for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean V/R			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**9500**	**500**	π_1	0.155 (0.003)	0.157 (0.003)	0.155 (0.003)	0.157 (0.003)
			π_2	0.146 (0.003)	0.140 (0.003)	0.146 (0.003)	0.141 (0.003)
			π_3	0.147 (0.003)	0.132 (0.003)	0.147 (0.003)	0.132 (0.003)
			π_4	0.147 (0.003)	0.122 (0.003)	0.147 (0.003)	0.122 (0.003)
			π_5	0.152 (0.003)	0.115 (0.003)	0.152 (0.003)	0.114 (0.003)
6	**5000**	**5000**	π_1	0.030 (<0.001)	0.030 (<0.001)	0.043 (<0.001)	0.043 (<0.001)
			π_2	0.050 (<0.001)	0.041 (<0.001)	0.074 (0.001)	0.064 (0.001)
			π_3	0.082 (0.001)	0.059 (0.001)	0.125 (0.001)	0.099 (0.001)
			π_4	0.136 (0.001)	0.080 (0.001)	0.205 (0.001)	0.146 (0.001)
			π_5	0.175 (0.001)	0.087 (0.001)	0.256 (0.001)	0.173 (0.002)
	7000	**3000**	π_1	0.046 (0.001)	0.046 (0.001)	0.053 (0.001)	0.054 (0.001)
			π_2	0.055 (0.001)	0.046 (0.001)	0.065 (0.001)	0.055 (0.001)
			π_3	0.066 (0.001)	0.045 (0.001)	0.080 (0.001)	0.057 (0.001)
			π_4	0.087 (0.001)	0.041 (0.001)	0.107 (0.001)	0.055 (0.001)
			π_5	0.100 (0.001)	0.034 (0.001)	0.125 (0.001)	0.048 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 11. The mean V/R for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean V/R			
n	**m_0**	**DE**	**π_i**	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**9000**	**1000**	**π_1**	0.088 (0.001)	0.089 (0.001)	0.089 (0.001)	0.089 (0.001)
			π_2	0.085 (0.001)	0.080 (0.001)	0.086 (0.001)	0.080 (0.001)
			π_3	0.090 (0.001)	0.077 (0.001)	0.091 (0.001)	0.077 (0.001)
			π_4	0.091 (0.001)	0.066 (0.001)	0.092 (0.001)	0.066 (0.001)
			π_5	0.092 (0.001)	0.061 (0.001)	0.093 (0.001)	0.061 (0.001)
	9500	**500**	**π_1**	0.120 (0.002)	0.121 (0.002)	0.120 (0.002)	0.121 (0.002)
			π_2	0.124 (0.002)	0.117 (0.002)	0.124 (0.002)	0.118 (0.002)
			π_3	0.121 (0.002)	0.114 (0.002)	0.121 (0.002)	0.114 (0.002)
			π_4	0.127 (0.002)	0.104 (0.002)	0.127 (0.002)	0.103 (0.002)
			π_5	0.125 (0.002)	0.097 (0.002)	0.125 (0.002)	0.097 (0.002)
10	**5000**	**5000**	**π_1**	0.028 (<0.001)	0.028 (<0.001)	0.044 (<0.001)	0.044 (<0.001)
			π_2	0.062 (<0.001)	0.049 (<0.001)	0.103 (0.001)	0.090 (0.001)
			π_3	0.114 (0.001)	0.080 (0.001)	0.192 (0.001)	0.164 (0.001)
			π_4	0.201 (0.001)	0.133 (0.001)	0.318 (0.001)	0.290 (0.001)
			π_5	0.258 (0.001)	0.170 (0.001)	0.382 (0.001)	0.361 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 11. The mean V/R for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean V/R			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**7000**	**3000**	π_1	0.044 (<0.001)	0.044 (<0.001)	0.053 (<0.001)	0.052 (<0.001)
			π_2	0.057 (0.001)	0.046 (0.001)	0.071 (0.001)	0.059 (0.001)
			π_3	0.077 (0.001)	0.048 (<0.001)	0.101 (0.001)	0.069 (0.001)
			π_4	0.108 (0.001)	0.048 (0.001)	0.148 (0.001)	0.077 (0.001)
			π_5	0.131 (0.001)	0.043 (0.001)	0.184 (0.001)	0.078 (0.001)
	9000	**1000**	π_1	0.074 (0.001)	0.074 (0.001)	0.075 (0.001)	0.075 (0.001)
			π_2	0.078 (0.001)	0.073 (0.001)	0.079 (0.001)	0.074 (0.001)
			π_3	0.081 (0.001)	0.068 (0.001)	0.082 (0.001)	0.068 (0.001)
			π_4	0.086 (0.001)	0.061 (0.001)	0.087 (0.001)	0.061 (0.001)
			π_5	0.089 (0.001)	0.055 (0.001)	0.092 (0.001)	0.055 (0.001)
	9500	**500**	π_1	0.107 (0.002)	0.108 (0.002)	0.107 (0.002)	0.108 (0.002)
			π_2	0.104 (0.002)	0.098 (0.002)	0.104 (0.002)	0.098 (0.002)
			π_3	0.107 (0.002)	0.097 (0.002)	0.107 (0.002)	0.097 (0.002)
			π_4	0.111 (0.002)	0.090 (0.002)	0.111 (0.002)	0.089 (0.002)
			π_5	0.104 (0.001)	0.080 (0.001)	0.104 (0.001)	0.078 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 11. The mean V/R for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean V/R			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**5000**	**5000**	π_1	0.028 (<0.001)	0.028 (<0.001)	0.044 (<0.001)	0.044 (<0.001)
			π_2	0.069 (<0.001)	0.054 (<0.001)	0.117 (0.001)	0.103 (0.001)
			π_3	0.133 (0.001)	0.095 (0.001)	0.229 (0.001)	0.206 (0.001)
			π_4	0.232 (0.001)	0.165 (0.001)	0.363 (0.001)	0.353 (0.001)
			π_5	0.296 (0.001)	0.219 (0.001)	0.423 (0.001)	0.421 (0.001)
	7000	**3000**	π_1	0.044 (0.001)	0.044 (0.001)	0.053 (0.001)	0.053 (<0.001)
			π_2	0.059 (<0.001)	0.047 (<0.001)	0.075 (0.001)	0.062 (0.001)
			π_3	0.085 (0.001)	0.052 (0.001)	0.113 (0.001)	0.078 (0.001)
			π_4	0.122 (0.001)	0.054 (0.001)	0.174 (0.001)	0.096 (0.001)
			π_5	0.148 (0.001)	0.049 (0.001)	0.215 (0.001)	0.103 (0.001)
	9000	**1000**	π_1	0.074 (0.001)	0.074 (0.001)	0.075 (0.001)	0.075 (0.001)
			π_2	0.076 (0.001)	0.070 (0.001)	0.077 (0.001)	0.071 (0.001)
			π_3	0.081 (0.001)	0.067 (0.001)	0.083 (0.001)	0.068 (0.001)
			π_4	0.086 (0.001)	0.060 (0.001)	0.088 (0.001)	0.060 (0.001)
			π_5	0.086 (0.001)	0.050 (0.001)	0.089 (0.001)	0.050 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 11. The mean V/R for proposed FDR methods using NBPSeq with associated standard errors in parentheses for each simulation setting (continued).

				NBPSeq			
				Mean V/R			
n	m_0	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**9500**	**500**	π_1	0.104 (0.002)	0.104 (0.002)	0.104 (0.002)	0.104 (0.002)
			π_2	0.102 (0.002)	0.097 (0.002)	0.102 (0.002)	0.097 (0.002)
			π_3	0.105 (0.001)	0.093 (0.001)	0.105 (0.002)	0.093 (0.001)
			π_4	0.105 (0.002)	0.087 (0.002)	0.105 (0.002)	0.086 (0.002)
			π_5	0.108 (0.002)	0.082 (0.001)	0.108 (0.002)	0.080 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

As seen in Table 12 (edgeR method), the traditional BH method did not outperform the asymmetric BH, traditional q-value or asymmetric q-value methods in any of the simulation settings in terms of mean *S.* The asymmetric BH method performed better than the traditional BH method in 64 of the 80 simulation settings with regard to mean *S* (16 of 20 settings with *n* = 4, 6, 10, and 12). The traditional q-value method performed better than the traditional BH method in 72 of 80 settings in terms of mean *S* (15 of 20 settings with *n* = 4, 17 of 20 settings with *n* = 6, and 20 of 20 settings with *n* = 10 and 12). Also, the asymmetric q-value method performed better than the traditional BH method in 76 of the 80 simulations settings with regard to mean *S* (19 of 20 settings with *n* = 4, 6, 10, and 12). Furthermore, the traditional q-value method performed better than the asymmetric BH method in 41 of 80 settings in terms of mean *S* (14 of 20 settings with *n* = 4, 9 of 20 settings with *n* = 6, 10 and 12). The asymmetric q-value method was never outperformed by the asymmetric BH method in 77 of 80 simulation settings in terms of mean *S.* Comparing the performance of the traditional q-value method to the asymmetric q-value method, the asymmetric q-value method performed better than the traditional q-value method in 68 of the 80 settings in terms of the mean *S* (16 of 20 settings with n = 4, 6, and 10, and 17 of 20 settings with n = 12).

Although a higher value of mean *S* was observed in most traditional BH method compared to the asymmetric BH method, in the 9 of 80 settings (n = 4 m0 = {7000, 9000} and π_1 , n = 6 and π_1, n =10 *m0* = {5000, 9500} and π_1, and n =12, *m0* = 7000 and π_1); these differences were not significant. Also, higher values of mean *S* were observed in the asymmetric BH method compared to the traditional BH method, there were no significant differences between these two methods in 4 of 80 settings (n = 10 *m0* = 700 and π_1, and n = 12 *m0* = {5000, 9000, 9500} and π_1). On the other hand, there was no significant difference in mean *S* at 5% between all methods with n = 4, *m0* = 9500 and π_1.

As shown in Table 13, the observed FDR (mean V/R) was comparable among all the methods, with levels elevated above 5%. Apart from simulation settings with π_1, the asymmetric BH and asymmetric q-value methods better controlled the observed FDR than the traditional BH and traditional q-value methods. In most settings, the traditional BH method compared to the asymmetric BH method, the asymmetric BH method better controlled the observed FDR close to or slightly higher than 5%.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting.

				edgeR			
				Mean S			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**5000**	**5000**	π_1	2100.580 (4.658)	2099.920 (4.692)	**2358.420** **(5.437)**	***2358.140*** ***(5.438)***
			π_2	1879.470 (4.938)	**1909.010** **(5.292)**	**2153.490** **(5.842)**	***2179.340*** ***(5.815)***
			π_3	1620.000 (4.759)	**1685.270** **(4.712)**	**1909.510** **(5.601)**	***1971.170*** ***(5.882)***
			π_4	1249.390 (4.336)	**1362.290** **(4.190)**	**1542.760** **(5.571)**	***1651.560*** ***(5.413)***
			π_5	1042.500 (4.421)	**1191.840** **(5.029)**	**1342.330** **(5.959)**	***1488.370*** ***(6.197)***
	7000	**3000**	π_1	1095.360 (3.243)	1094.530 (3.287)	**1154.360** **(3.604)**	***1154.180*** ***(3.664)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**7000**	**3000**	π_2	1046.320 (3.856)	**1069.200** **(3.917)**	**1112.200** **(4.110)**	***1131.210*** ***(4.103)***
			π_3	969.970 (3.871)	**1019.400** **(3.950)**	**1036.710** **(4.193)**	***1083.080*** ***(3.891)***
			π_4	853.090 (3.503)	**953.580** **(3.600)**	**924.490** **(3.957)**	***1016.600*** ***(3.910)***
			π_5	777.220 (3.439)	**907.650** **(3.817)**	**849.250** **(3.927)**	***969.100*** ***(3.964)***
	9000	**1000**	π_1	272.130 (1.897)	272.130 (1.904)	**272.920** **(1.925)**	***273.300*** ***(1.904)***
			π_2	267.940 (2.009)	**274.450** **(1.948)**	**268.620** **(2.024)**	**274.720** **(1.980)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m_0***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**9000**	**1000**	π_3	259.520 (1.610)	**275.960** **(1.610)**	**260.330** **(1.634)**	**275.850** **(1.642)**
			π_4	252.260 (1.861)	**283.780** **(1.968)**	**253.180** **(1.888)**	**283.100** **(2.001)**
			π_5	251.470 (1.858)	**292.820** **(1.857)**	**252.440** **(1.888)**	**291.810** **(1.884)**
	9500	**500**	π_1	110.680 (1.172)	110.990 (1.186)	110.730 (1.176)	110.850 (1.171)
			π_2	114.320 (1.190)	**117.350** **(1.254)**	114.360 (1.193)	**117.240** **(1.248)**
			π_3	113.970 (1.207)	**121.390** **(1.221)**	113.980 (1.206)	**121.200** **(1.23)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m*₀**	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**9500**	**500**	$\boldsymbol{\pi_4}$	110.440 (1.383)	**124.000** **(1.396)**	110.470 (1.383)	**123.590** **(1.396)**
			$\boldsymbol{\pi_5}$	109.300 (1.378)	**127.520** **(1.416)**	109.340 (1.375)	**127.000** **(1.404)**
6	**5000**	**5000**	$\boldsymbol{\pi_1}$	2833.860 (4.548)	2833.360 (4.565)	**3072.530** **(4.835)**	***3072.470*** ***(4.835)***
			$\boldsymbol{\pi_2}$	2595.670 (4.276)	**2633.940** **(4.380)**	**2883.080** **(5.307)**	***2910.790*** ***(5.287)***
			$\boldsymbol{\pi_3}$	2291.410 (3.813)	**2370.960** **(4.014)**	**2641.800** **(4.380)**	***2697.600*** ***(4.592)***
			$\boldsymbol{\pi_4}$	1887.380 (4.767)	**2014.910** **(5.109)**	**2299.340** **(5.945)**	***2395.980*** ***(5.734)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**5000**	**5000**	π_5	1655.300 (4.433)	**1813.270** **(4.592)**	**2109.320** **(5.908)**	***2220.380*** ***(5.689)***
	7000	**3000**	π_1	1559.790 (3.380)	1559.720 (3.432)	**1621.810** **(3.427)**	***1621.480*** ***(3.442)***
			π_2	1496.170 (3.596)	**1518.850** **(3.426)**	**1566.060** **(3.801)**	***1583.690*** ***(2.941)***
			π_3	1398.440 (3.261)	**1456.750** **(3.262)**	**1480.940** **(3.241)**	***1527.280*** ***(3.649)***
			π_4	1274.760 (3.250)	**1377.080** **(3.183)**	**1367.020** **(3.404)**	***1450.690*** ***(3.311)***
			π_5	1198.010 (3.382)	**1333.240** **(3.264)**	**1296.030** **(3.627)**	***1408.980*** ***(3.482)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**9000**	**1000**	π_1	427.430 (2.029)	427.200 (2.027)	**428.660** **(2.040)**	***428.270*** ***(2.046)***
			π_2	423.990 (1.975)	**430.410** **(1.940)**	**425.520** **(1.996)**	***431.520*** ***(1.966)***
			π_3	413.320 (1.814)	**429.890** **(1.913)**	**414.720** **(1.821)**	***430.300*** ***(1.950)***
			π_4	407.440 (1.909)	**437.120** **(1.952)**	**409.220** **(1.907)**	**436.200** **(1.960)**
			π_5	402.360 (1.853)	**442.150** **(1.914)**	**404.400** **(1.899)**	**441.170** **(1.930)**
	9500	**500**	π_1	190.350 (1.526)	190.210 (1.543)	**190.400** **(1.521)**	190.370 (1.533)

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m₀***	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**9500**	**500**	$\boldsymbol{\pi_2}$	189.170 (1.350)	**192.080** **(1.338)**	189.170 (1.350)	**192.060** **(1.340)**
			$\boldsymbol{\pi_3}$	184.740 (1.364)	**192.190** **(1.323)**	**184.770** **(1.366)**	**191.740** **(1.315)**
			$\boldsymbol{\pi_4}$	185.200 (1.252)	**198.790** **(1.212)**	185.260 (1.255)	**198.430** **(1.210)**
			$\boldsymbol{\pi_5}$	184.990 (1.201)	**203.250** **(1.271)**	185.050 (1.201)	**202.100** **(1.284)**
10	**5000**	**5000**	$\boldsymbol{\pi_1}$	3541.010 (4.031)	3540.830 (4.009)	**3732.970** **(3.754)**	***3732.940*** ***(3.764)***
			$\boldsymbol{\pi_2}$	3292.040 (3.948)	**3336.910** **(3.985)**	**3575.120** **(4.396)**	***3598.460*** ***(4.525)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m*₀**	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**5000**	**5000**	$\boldsymbol{\pi_3}$	3000.480 (3.497)	**3082.070** **(3.724)**	**3392.540** **(4.501)**	***3432.650*** ***(4.630)***
			$\boldsymbol{\pi_4}$	2622.760 (3.209)	**2740.02** **(3.28)**	**3178.550** **(4.934)**	***3217.380*** ***(4.705)***
			$\boldsymbol{\pi_5}$	2400.130 (3.487)	**2523.180** **(3.574)**	**3045.570** **(5.067)**	***3080.840*** ***(4.817)***
	7000	**3000**	$\boldsymbol{\pi_1}$	2016.610 (2.865)	2016.730 (2.853)	**2070.450** **(2.950)**	***2070.210*** ***(2.931)***
			$\boldsymbol{\pi_2}$	1950.220 (3.381)	**1973.910** **(3.395)**	**2016.210** **(3.475)**	***2031.980*** ***(3.383)***
			$\boldsymbol{\pi_3}$	1862.010 (2.750)	**1917.520** **(2.799)**	**1944.790** **(2.943)**	***1980.600*** ***(2.980)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**5000**	**5000**	π_3	3000.480 (3.497)	**3082.070** **(3.724)**	**3392.540** **(4.501)**	***3432.650*** ***(4.630)***
			π_4	2622.760 (3.209)	**2740.02** **(3.28)**	**3178.550** **(4.934)**	***3217.380*** ***(4.705)***
			π_5	2400.130 (3.487)	**2523.180** **(3.574)**	**3045.570** **(5.067)**	***3080.840*** ***(4.817)***
	7000	**3000**	π_1	2016.610 (2.865)	2016.730 (2.853)	**2070.450** **(2.950)**	***2070.210*** ***(2.931)***
			π_2	1950.220 (3.381)	**1973.910** **(3.395)**	**2016.210** **(3.475)**	***2031.980*** ***(3.383)***
			π_3	1862.010 (2.750)	**1917.520** **(2.799)**	**1944.790** **(2.943)**	***1980.600*** ***(2.980)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**7000**	**3000**	π_4	1740.210 (2.734)	**1835.260** **(2.929)**	**1849.880** **(3.019)**	***1909.720*** ***(2.988)***
			π_5	1662.770 (3.226)	**1784.100** **(3.212)**	**1788.810** **(3.395)**	***1867.550*** ***(3.348)***
	9000	**1000**	π_1	601.180 (1.915)	601.180 (1.923)	**603.200** **(1.916)**	***603.090*** ***(1.941)***
			π_2	593.500 (1.860)	**598.110** **(1.825)**	**595.690** **(1.880)**	***599.360*** ***(1.866)***
			π_3	589.650 (1.711)	**603.220** **(1.590)**	**592.290** **(1.732)**	***603.830*** ***(1.647)***
			π_4	576.600 (1.908)	**600.760** **(1.835)**	**579.590** **(1.939)**	**599.890** **(1.894)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**9000**	**1000**	π_5	569.330 (1.717)	**602.860** **(1.651)**	**572.940** **(1.739)**	**601.860** **(1.654)**
	9500	**500**	π_1	278.460 (1.157)	278.400 (1.154)	**278.500** **(1.158)**	*278.560* *(1.162)*
			π_2	278.040 (1.233)	**281.150** **(1.267)**	**278.260** **(1.211)**	**281.160** **(1.256)**
			π_3	275.600 (1.174)	**281.110** **(1.173)**	**275.720** **(1.174)**	**280.730** **(1.164)**
			π_4	273.110 (1.245)	**284.570** **(1.181)**	**273.250** **(1.242)**	**283.780** **(1.165)**
			π_5	272.200 (1.148)	**287.730** **(1.157)**	**272.310** **(1.149)**	**287.630** **(1.177)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m_0***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**5000**	**5000**	π_1	3735.490 (3.453)	3735.550 (3.477)	**3909.540** **(3.352)**	***3909.540*** ***(3.354)***
			π_2	3503.510 (3.244)	**3546.260** **(3.160)**	**3771.940** **(3.224)**	***3793.020*** ***(3.237)***
			π_3	3221.560 (3.088)	**3302.500** **(3.213)**	**3628.670** **(4.198)**	***3656.110*** ***(4.184)***
			π_4	2850.340 (3.701)	**2952.850** **(3.695)**	**3456.240** **(5.184)**	***3474.640*** ***(4.9212)***
			π_5	2649.000 (3.521)	**2755.540** **(3.493)**	**3358.700** **(5.945)**	***3367.940*** ***(5.572)***
	7000	**3000**	π_1	2145.130 (2.580)	2145.060 (2.579)	**2193.500** **(2.698)**	***2193.690*** ***(2.688)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**7000**	**3000**	π_2	2082.840 (2.553)	**2106.650** **(2.428)**	**2144.910** **(2.657)**	***2159.300*** ***(2.595)***
			π_3	2006.030 (2.701)	**2056.890** **(2.522)**	**2088.100** **(2.830)**	***2118.430*** ***(2.591)***
			π_4	1882.080 (3.314)	**1973.600** **(2.852)**	**1997.450** **(3.155)**	***2050.680*** ***(2.925)***
			π_5	1812.500 (2.934)	**1926.300** **(2.712)**	**1948.750** **(2.957)**	***2014.390*** ***(3.115)***
	9000	**1000**	π_1	651.320 (1.513)	651.380 (1.507)	**653.720** **(1.463)**	***653.540*** ***(1.472)***
			π_2	645.090 (1.652)	**651.160** **(1.664)**	**647.700** **(1.678)**	***652.370*** ***(1.638)***

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m_0***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**9000**	**1000**	π_3	639.270 (1.768)	**651.110** **(1.723)**	**641.860** **(1.763)**	***651.640*** ***(1.743)***
			π_4	627.890 (1.675)	**651.950** **(1.670)**	**631.420** **(1.707)**	**651.370** **(1.698)**
			π_5	619.870 (1.597)	**651.920** **(1.706)**	**624.060** **(1.656)**	**650.540** **(1.710)**
	9500	**500**	π_1	307.110 (1.256)	307.150 (1.253)	**307.280** **(1.254)**	307.310 (1.260)
			π_2	305.330 (1.273)	**307.690** **(1.214)**	**305.660** **(1.276)**	**307.740** **(1.236)**
			π_3	304.630 (1.203)	**309.670** **(1.214)**	**304.700** **(1.208)**	**309.350** **(1.207)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 12. The mean S for proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean S			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**9500**	**500**	π_4	299.080 (1.264)	**309.450** **(1.263)**	**299.380** **(1.261)**	**308.810** **(1.261)**
			π_5	301.270 (1.345)	**315.500** **(1.336)**	**301.620** **(1.352)**	**315.810** **(1.339)**

For each setting, the significant higher mean *S* value at 5% significance level is shown in bolded fonts. If the traditional QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is underlined. If the asymmetric QV method has a significant higher mean *S* compared to the asymmetric BH method, then the mean *S* is *italicized*. Also, if the asymmetric QV method has a significant higher mean *S* compared to the traditional QV method, then the mean *S* is underlined. The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 13. The mean V/R proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting.

				edgeR			
				Mean V/R			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**5000**	**5000**	π_1	0.029 (<0.001)	0.029 (<0.001)	0.041 (<0.001)	0.041 (<0.001)
			π_2	0.043 (<0.001)	0.037 (<0.001)	0.062 (0.001)	0.055 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 13. The mean V/R proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean V/R			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**5000**	**5000**	π_3	0.065 (0.001)	0.049 (0.001)	0.092 (0.001)	0.071 (0.001)
			π_4	0.102 (0.001)	0.060 (0.001)	0.142 (0.001)	0.090 (0.001)
			π_5	0.133 (0.001)	0.059 (0.001)	0.182 (0.001)	0.094 (0.001)
	7000	**3000**	π_1	0.046 (0.001)	0.047 (0.001)	0.053 (0.001)	0.053 (0.001)
			π_2	0.053 (0.001)	0.046 (0.001)	0.061 (0.001)	0.053 (0.001)
			π_3	0.060 (0.001)	0.042 (0.001)	0.070 (0.001)	0.050 (0.001)
			π_4	0.074 (0.001)	0.038 (0.001)	0.087 (0.001)	0.045 (0.001)
			π_5	0.081 (0.001)	0.029 (0.001)	0.096 (0.001)	0.036 (0.001)
	9000	**1000**	π_1	0.088 (0.002)	0.088 (0.002)	0.088 (0.002)	0.089 (0.002)
			π_2	0.088 (0.002)	0.082 (0.001)	0.089 (0.002)	0.082 (0.001)
			π_3	0.091 (0.002)	0.077 (0.001)	0.091 (0.002)	0.077 (0.001)
			π_4	0.093 (0.002)	0.069 (0.001)	0.094 (0.002)	0.069 (0.001)
			π_5	0.094 (0.002)	0.065 (0.001)	0.095 (0.002)	0.066 (0.001)
	9500	**500**	π_1	0.127 (0.003)	0.127 (0.003)	0.127 (0.003)	0.127 (0.003)
			π_2	0.125 (0.003)	0.121 (0.003)	0.125 (0.003)	0.121 (0.003)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 13. The mean V/R proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean V/R			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
4	**9500**	**500**	π_3	0.122 (0.003)	0.111 (0.003)	0.122 (0.003)	0.111 (0.003)
			π_4	0.122 (0.003)	0.101 (0.003)	0.122 (0.003)	0.101 (0.003)
			π_5	0.125 (0.003)	0.095 (0.003)	0.125 (0.003)	0.095 (0.003)
6	**5000**	**5000**	π_1	0.030 (<0.001)	0.030 (<0.001)	0.044 (<0.001)	0.044 (<0.001)
			π_2	0.051 (<0.001)	0.043 (<0.001)	0.077 (<0.001)	0.068 (0.001)
			π_3	0.085 (0.001)	0.063 (0.001)	0.130 (0.001)	0.104 (0.001)
			π_4	0.142 (0.001)	0.086 (0.001)	0.209 (0.001)	0.153 (0.001)
			π_5	0.182 (0.001)	0.095 (0.001)	0.260 (0.001)	0.181 (0.002)
	7000	**3000**	π_1	0.046 (0.001)	0.046 (0.001)	0.054 (0.001)	0.054 (0.001)
			π_2	0.055 (0.001)	0.046 (0.001)	0.065 (0.001)	0.056 (0.001)
			π_3	0.065 (0.001)	0.044 (0.001)	0.080 (0.001)	0.056 (0.001)
			π_4	0.085 (0.001)	0.040 (0.001)	0.106 (0.001)	0.053 (0.001)
			π_5	0.097 (0.001)	0.033 (0.001)	0.122 (0.001)	0.046 (0.001)
	9000	**1000**	π_1	0.080 (0.001)	0.080 (0.001)	0.081 (0.001)	0.081 (0.001)
			π_2	0.081 (0.001)	0.075 (0.001)	0.082 (0.001)	0.076 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 13. The mean V/R proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean V/R			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
6	**9000**	**1000**	π_3	0.083 (0.001)	0.070 (0.001)	0.084 (0.001)	0.071 (0.001)
			π_4	0.083 (0.001)	0.061 (0.001)	0.085 (0.001)	0.060 (0.001)
			π_5	0.086 (0.001)	0.056 (0.001)	0.087 (0.001)	0.056 (0.001)
	9500	**500**	π_1	0.104 (0.002)	0.103 (0.002)	0.104 (0.002)	0.104 (0.002)
			π_2	0.104 (0.002)	0.101 (0.002)	0.105 (0.002)	0.101 (0.002)
			π_3	0.103 (0.002)	0.097 (0.002)	0.104 (0.002)	0.096 (0.002)
			π_4	0.109 (0.002)	0.089 (0.002)	0.109 (0.002)	0.089 (0.002)
			π_5	0.107 (0.002)	0.082 (0.002)	0.107 (0.002)	0.081 (0.002)
10	**5000**	**5000**	π_1	0.029 (<0.001)	0.029 (<0.001)	0.047 (<0.001)	0.047 (<0.001)
			π_2	0.067 (<0.001)	0.053 (<0.001)	0.110 (0.001)	0.097 (0.001)
			π_3	0.120 (0.001)	0.087 (0.001)	0.194 (0.001)	0.167 (0.001)
			π_4	0.209 (0.001)	0.146 (0.001)	0.316 (0.001)	0.288 (0.001)
			π_5	0.264 (0.001)	0.184 (0.001)	0.376 (0.001)	0.354 (0.001)
	7000	**3000**	π_1	0.044 (<0.001)	0.044 (<0.001)	0.054 (0.001)	0.054 (0.001)
			π_2	0.059 (0.001)	0.047 (0.001)	0.074 (0.001)	0.063 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 13. The mean V/R proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean V/R			
n	***m*₀**	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
10	**7000**	**3000**	π_3	0.079 (0.001)	0.050 (0.001)	0.102 (0.001)	0.071 (0.001)
			π_4	0.109 (0.001)	0.048 (0.001)	0.146 (0.001)	0.077 (0.001)
			π_5	0.130 (0.001)	0.043 (0.001)	0.177 (0.001)	0.074 (0.001)
	9000	**1000**	π_1	0.069 (0.001)	0.069 (0.001)	0.071 (0.001)	0.071 (0.001)
			π_2	0.072 (0.001)	0.067 (0.001)	0.074 (0.001)	0.069 (0.001)
			π_3	0.075 (0.001)	0.061 (0.001)	0.077 (0.001)	0.062 (0.001)
			π_4	0.080 (0.001)	0.054 (0.001)	0.083 (0.001)	0.055 (0.001)
			π_5	0.083 (0.001)	0.048 (0.001)	0.086 (0.001)	0.049 (0.001)
	9500	**500**	π_1	0.089 (0.002)	0.089 (0.002)	0.089 (0.002)	0.089 (0.002)
			π_2	0.088 (0.002)	0.083 (0.002)	0.089 (0.002)	0.083 (0.002)
			π_3	0.092 (0.002)	0.082 (0.002)	0.092 (0.002)	0.082 (0.002)
			π_4	0.094 (0.002)	0.075 (0.002)	0.094 (0.002)	0.074 (0.002)
			π_5	0.091 (0.002)	0.066 (0.001)	0.091 (0.002)	0.063 (0.001)
12	**5000**	**5000**	π_1	0.029 (<0.001)	0.029 (<0.001)	0.047 (<0.001)	0.047 (<0.001)
			π_2	0.074 (<0.001)	0.058 (<0.001)	0.123 (0.001)	0.109 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 13. The mean V/R proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean V/R			
n	***m₀***	**DE**	π_i	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**5000**	**5000**	π_3	0.140 (0.001)	0.103 (0.001)	0.230 (0.001)	0.208 (0.001)
			π_4	0.240 (0.001)	0.179 (0.001)	0.360 (0.001)	0.347 (0.001)
			π_5	0.300 (0.001)	0.232 (0.001)	0.416 (0.001)	0.411 (0.001)
	7000	**3000**	π_1	0.044 (<0.001)	0.044 (<0.001)	0.055 (<0.001)	0.055 (0.001)
			π_2	0.060 (0.001)	0.047 (<0.001)	0.078 (0.001)	0.065 (0.001)
			π_3	0.087 (0.001)	0.054 (0.001)	0.115 (0.001)	0.080 (0.001)
			π_4	0.122 (0.001)	0.055 (0.001)	0.169 (0.001)	0.095 (0.001)
			π_5	0.146 (0.001)	0.049 (0.001)	0.204 (0.001)	0.097 (0.001)
	9000	**1000**	π_1	0.068 (0.001)	0.068 (0.001)	0.070 (0.001)	0.070 (0.001)
			π_2	0.070 (0.001)	0.064 (0.001)	0.072 (0.001)	0.065 (0.001)
			π_3	0.075 (0.001)	0.060 (0.001)	0.077 (0.001)	0.062 (0.001)
			π_4	0.080 (0.001)	0.054 (0.001)	0.083 (0.001)	0.055 (0.001)
			π_5	0.081 (0.001)	0.045 (0.001)	0.085 (0.001)	0.046 (0.001)
	9500	**500**	π_1	0.086 (0.001)	0.086 (0.001)	0.086 (0.002)	0.087 (0.001)
			π_2	0.083 (0.001)	0.078 (0.001)	0.083 (0.001)	0.079 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

Table 13. The mean V/R proposed FDR methods using edgeR with associated standard errors in parentheses for each simulation setting (continued).

				edgeR			
				Mean V/R			
n	***m*₀**	**DE**	$\boldsymbol{\pi_i}$	**Traditional BH**	**Asymmetric BH**	**Traditional QV**	**Asymmetric QV**
12	**9500**	**500**	$\boldsymbol{\pi_3}$	0.087 (0.002)	0.078 (0.002)	0.087 (0.001)	0.078 (0.002)
			$\boldsymbol{\pi_4}$	0.087 (0.002)	0.071 (0.001)	0.088 (0.001)	0.070 (0.001)
			$\boldsymbol{\pi_5}$	0.092 (0.002)	0.067 (0.001)	0.092 (0.002)	0.062 (0.001)

The π_i's represent the proportion of DE genes that are upregulated and downregulated. $\pi_1 = (0.5,\ 0.5)$, $\pi_2 = (0.7,\ 0.3)$, $\pi_3 = (0.8,\ 0.2)$, $\pi_4 = (0.9,\ 0.1)$ and $\pi_5 = (0.95,\ 0.05)$.

5.6. Real data analysis

In this section, RNA-Seq data from a real gene expression experiment described by Bottomly et al. (2011) is reanalyzed using the traditional and asymmetric BH methods, and the traditional and asymmetric q-value methods for the DESeq2, NBPSeq, and edgeR methods. The description of the data was previously discussed in section 3.5. The data consist of two classes (B6 and D2); with a total of n = 21 samples, n_1 = 10 B6 samples and n_2 = 11 D2 samples. The data set contains 36,536 genes, the total number of genes m = 13,932 were analyzed after filtering to remove genes without any reads.

The number of genes declared to be DE using all methods for estimating FDR (traditional and asymmetric BH and traditional and asymmetric q-value) for DESeq2, NBPSeq, and edgeR methods while controlling FDR at 5% are summarized in Figure 5, 6 and 7 respectively below. The total number of genes declared to be DE using all FDR methods for DESeq2, NBPSeq and edgeR are summarized in Table 14, 15, 16 respectively below.

The analysis was performed on a real, not simulated, data set, therefore genes that are EE and DE are not known. Thus, evaluating the true FDR associated with each method cannot be done. However, because the sample size for each class is relatively large with a small degree of asymmetry, the estimation of the FDR is being adequately controlled at 5% based on the results of the simulation study in section 5.4.

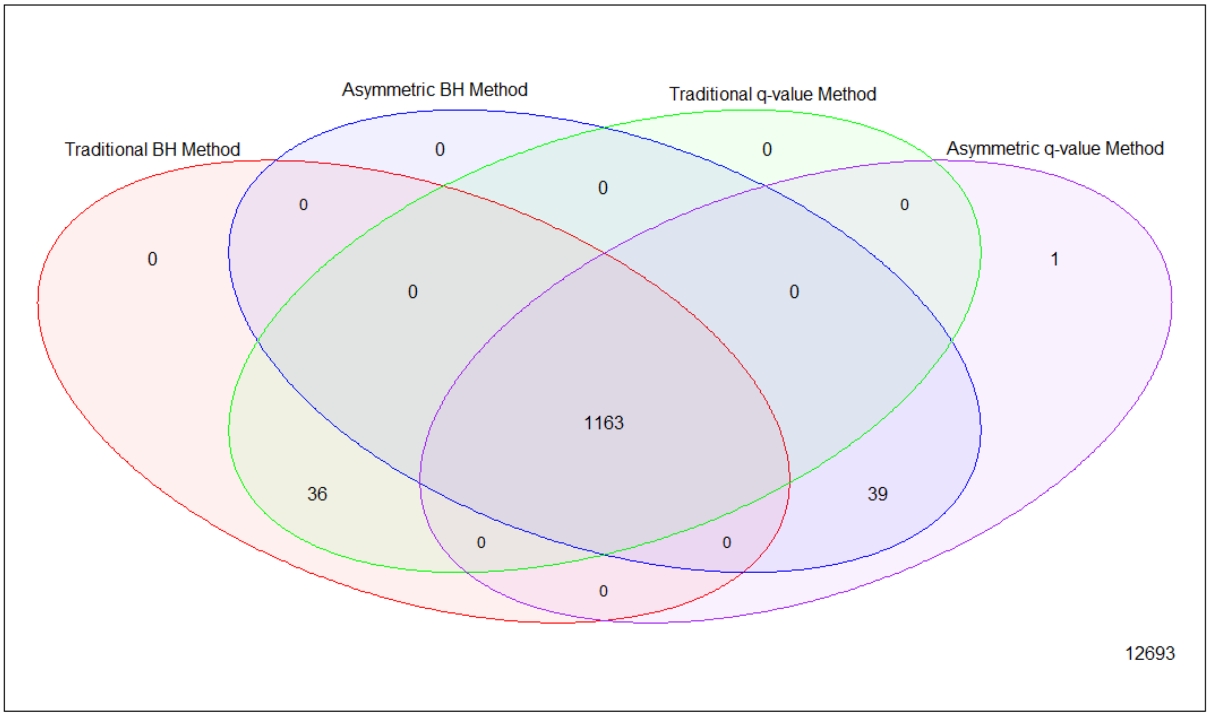

Figure 5. Venn diagram of genes declared to be DE for DESeq2 method using all FDR methods.

There were 1163 genes that were DDE by all methods. The asymmetric q-value method declared 1 more gene to be DE. The asymmetric q-value method declared more genes to be DE. This is not surprising based on the results from the simulation studies in section 5.4.

Table 14. Total number of genes declared to be differentially expressed using all FDR methods for DESeq2 method.

Method	Total number of genes DDE
Traditional BH	1199
Asymmetric BH	1199
Traditional q-value	1202
Asymmetric q-value	1203

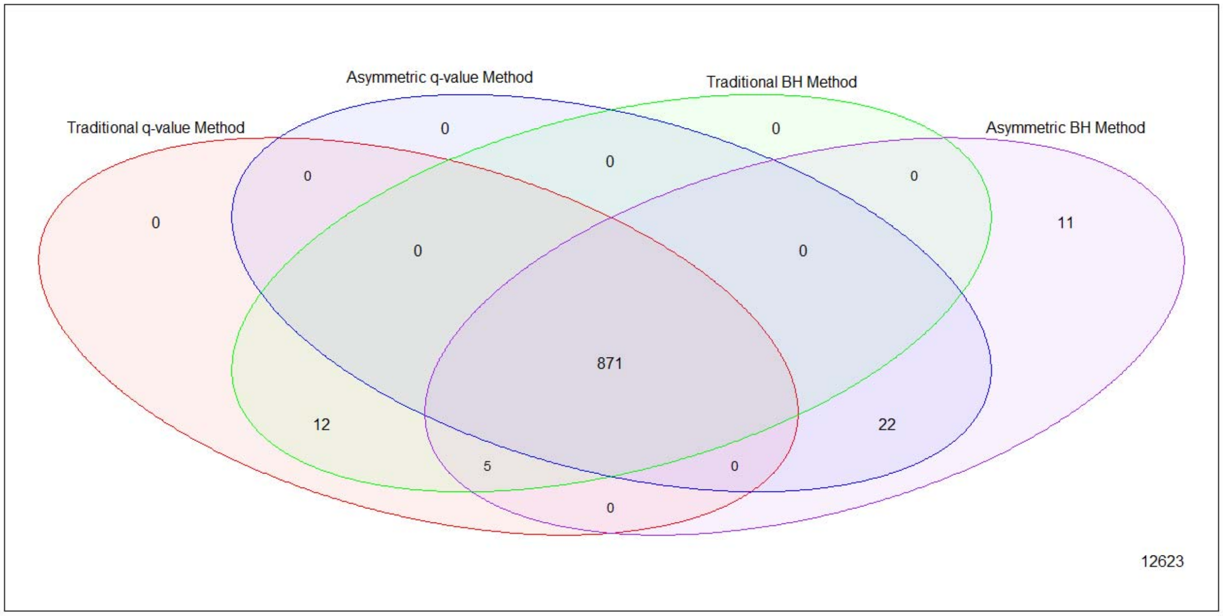

Figure 6. Venn diagram of genes declared to be DE for NBPSeq method using all FDR methods.

There were 871 genes that were DDE by all methods. An additional 12 genes were DDE by the traditional q-value and BH methods. Both the asymmetric BH and q-value methods declared 22 more genes to be DE. Asymmetric BH method declared additional 11 genes to be DE. Hence, both the asymmetric q-value and BH methods declared the most genes to be DE, this is not surprising based on the results from the simulation studies in section 5.4.

Table 15. Total number of genes declared to be differentially expressed using all FDR methods for NBPSeq method.

Method	Total number of genes DDE
Traditional q-value	888
Asymmetric q-value	893
Traditional BH	888
Asymmetric BH	909

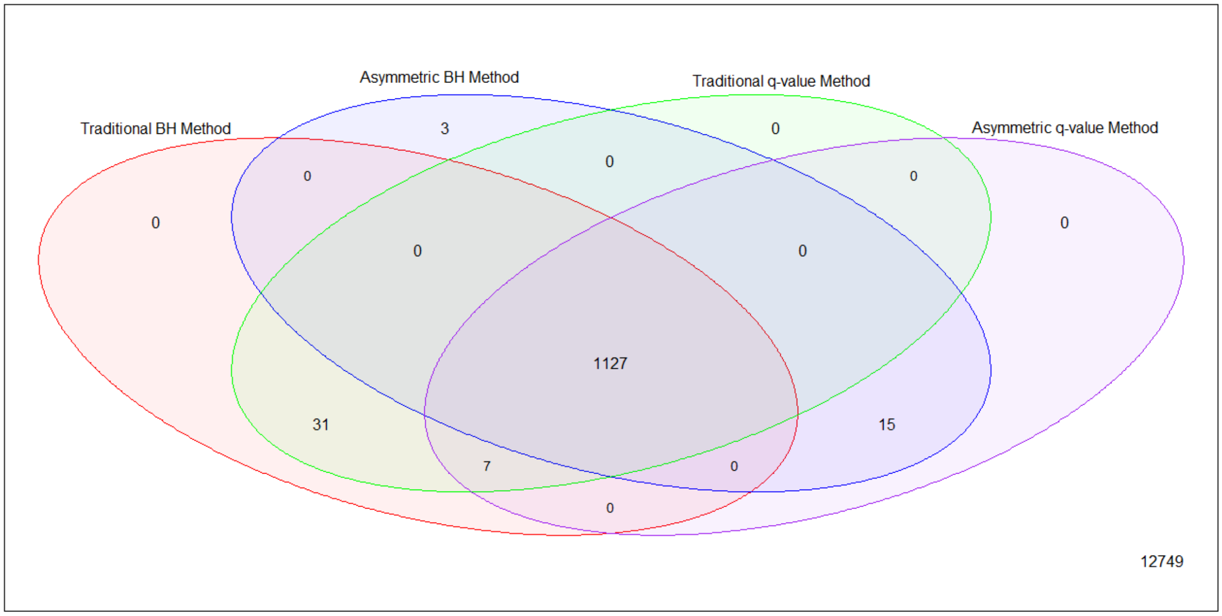

Figure 7. Venn diagram of genes declared to be DE for edgeR method using all FDR methods.

There were 1127 genes that were DDE by all methods. Additional 31 genes were DDE by both the traditional BH and q-value methods. Both the asymmetric BH and q-value methods declared 15 more genes to be DE. Asymmetric BH method declared additional 3 genes to be DE. Hence, both the traditional BH and q-value methods declared the most genes to be DE, this is not surprising based on the results from the simulation studies in section 5.4.

Table 16 Total number of genes declared to be differentially expressed using all FDR methods for the edgeR method.

Method	**Total number of genes DDE**
Traditional BH	1165
Asymmetric BH	1145
Traditional q-value	1165
Asymmetric q-value	1149

5.7. Discussion

The asymmetric BH and q-value methods for estimating FDR, when there exists asymmetry in the distribution of the test statistics, has observed advantages over the traditional BH and q-value methods. The observed FDRs for DESeq2, NBPSeq, and edgeR were elevated in most of these settings where the degree of asymmetry was high (80%, 90%, and 95% of genes upregulated). For DESeq2 method, using the asymmetric BH or q-value methods is recommended but preferably, the asymmetric q-value method should be used to estimate FDR when the degree of asymmetry is high. The asymmetric q-value method should be used to estimate FDR for the NBPseq method rather than the traditional q-value method. For the edgeR method, using the asymmetric BH and q-value methods is recommended, but preferably, the asymmetric BH method should be used when the degree of asymmetry is high. When the estimated percentage of EE genes is high and the proportion of genes that are upregulated and downregulated are the same, use of the original methods used to estimate FDR are recommended for DESeq2, NBPSeq, and edgeR.

Using real RNA-Seq data, the traditional and asymmetric and q-value methods declared more genes to be DE than the other methods at 5% significance level for DESeq2, which is consistent with the simulation results. Asymmetric BH and q-value methods declared more genes to be DE than the other methods at 5% significance level for NBPSeq. For edgeR, traditional BH and q-value methods declared more genes to be DE than the other methods at 5% significance level.

CHAPTER 6. CONCLUSION

The performance of proposed methods I and II that takes into account asymmetry found in the distribution of the effect sizes in Chapters 3 and 4 indicates that the observed FDR was adequately controlled for larger sample sizes (n = 6, 10, 12) and when the degree of asymmetry is high (80%, 90%, and 95% of genes upregulated). In terms of the mean *S* from the simulation studies and the number of genes declared to DE using real gene expression experiment, the proposed methods I and II identified and declared more genes to DE compared to the traditional method (SAMseq). For smaller sample sizes, the SAMseq method and proposed methods I and II are not recommended. Other commonly-used methods such DESeq2, NBPSeq, and edgeR methods should be used.

For any analysis where the distribution of the data is unknown, proposed methods I and II should be used over the other methods evaluated in this paper. Preferably, proposed method II should be used since it controls the observed FDR better than the other methods compared in this research and has higher power than proposed method I. Also, the probability of type 1 error was not compared. There is the possibility that proposed methods I and II could have higher probability of type 1 error compared to other commonly-used methods; however, this was not investigated because FDR is a more appropriate error rate to control in gene expression experiments, and FDR was adequately controlled for all sample sizes except sample size of four ($n = 4$) for both proposed methods.

The performance of all the methods used to estimate FDR (traditional BH method, asymmetric BH method, traditional q-value method and asymmetric q-value method) in Chapter 5 indicates that the observed FDR was not adequately controlled at 5% significance level when the degree of asymmetry was high (80%, 90%, and 95% of genes upregulated) in most simulation settings. In simulation settings where the degree of asymmetry was low (50%, and 70% of genes upregulated), all methods used to estimate the observed FDRs for DESeq2, NBPSeq and edgeR were adequately controlled close to 5% significance level.

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APPENDIX. R CODE

```
library(samr)
library(impute)


## Note: parts of the samr functions, modified to implements proposed method
I and II
##functions
##sequencing depth
seq.depth <- function(x) {
      iter <- 5
      cmeans <- colSums(x)/sum(x)
      for (i in 1:iter) {
            n0 <- rowSums(x) %*% t(cmeans)
            prop <- rowSums((x - n0)^2/(n0 + 1e-08))
            qs <- quantile(prop, c(0.25, 0.75))
            keep <- (prop >= qs[1]) & (prop <= qs[2])
            cmeans <- colMeans(x[keep, ])
            cmeans <- cmeans/sum(cmeans)
      }
      depth <- cmeans/mean(cmeans)
      return(depth)
}


##ranking within column (function to rank the data within #column)
rankcol <- function(x) {
      # ranks the elements within each col of the matrix x
      # and returns these ranks in a matrix
      n <- nrow(x)
      p <- ncol(x)
      mode(n) <- "integer"
      mode(p) <- "integer"
      mode(x) <- "single"
      if (!is.loaded("rankcol")) {
            #dyn.load('/home/tibs/PAPERS/jun2/test/rankcol.so')
      }
      junk = .Fortran("rankcol", x, n, p, xr = integer(n * p),
                  integer(n), PACKAGE = "samr")
      xr = matrix(junk$xr, nrow = n, ncol = p)
      return(xr)
      }


##resampling of the data
resample <- function(x, d, nresamp = 20) {
      ng <- nrow(x)
      ns <- ncol(x)
      dbar <- exp(mean(log(d)))
      xresamp <- array(0, dim = c(ng, ns, nresamp))
      for (k in 1:nresamp) {
            for (j in 1:ns) {
                  xresamp[, j, k] <- rpois(n = ng, lambda = (dbar/d[j]) * x[,
                  j]) + runif(ng) * 0.1
            }
```

```
      }
      for (k in 1:nresamp) {
            xresamp[, , k] <- t(rankcol(t(xresamp[, , k])))
      }
      return(xresamp)
      }


##test statistic (Wilcoxon two class unpaired)
##ordered test statistic with its rank
wilcoxon.unpaired.seq.func <- function(xresamp, y) {
            tt <- rep(0, dim(xresamp)[1])
            for (i in 1:dim(xresamp)[3]) {
                  tt <- tt + rowSums(xresamp[, y == 2, i]) - sum(y == 2) *
                  (length(y) + 1)/2
            }
            tt <- tt/dim(xresamp)[3]
            or.tt <- sort(tt,decreasing=FALSE)
            rk.tt <- rank(tt)

            return(list(tt = tt, ordered.tt = or.tt, rank.tt = rk.tt ))
      }


##permuted test statistics
insert.value <- function(vec, newval, pos) {
      if (pos == 1)
            return(c(newval, vec))
      lvec <- length(vec)
      if (pos > lvec)
            return(c(vec, newval))
      return(c(vec[1:pos - 1], newval, vec[pos:lvec]))
}

permute <- function(elem) {
      # generates all perms of the vector elem
      if (!missing(elem)) {
            if (length(elem) == 2)
              return(matrix(c(elem, elem[2], elem[1]), nrow = 2))
           last.matrix <- permute(elem[-1])
           dim.last <- dim(last.matrix)
           new.matrix <- matrix(0, nrow = dim.last[1] *
      (dim.last[2] + 1), ncol = dim.last[2] + 1)

           for (row in 1:(dim.last[1])) {
               for (col in 1:(dim.last[2] + 1)) new.matrix[row +
                      (col - 1) * dim.last[1], ] <-
                  insert.value(last.matrix[row, ],
                  elem[1], col)
           }
           return(new.matrix)
      }
      else cat("Usage: permute(elem)\n\twhere elem is a
      vector\n")
      }

getperms <- function(y, nperms) {
```

```
            total.perms = factorial(length(y))
            if (total.perms <= nperms) {
                  perms = permute(1:length(y))
                  all.perms.flag = 1
                  nperms.act = total.perms
            }
            if (total.perms > nperms) {
                  perms = matrix(NA, nrow = nperms, ncol =
            length(y))
                  for (i in 1:nperms) {
                        perms[i, ] = sample(1:length(y), size =
            length(y))
                  }
                  all.perms.flag = 0
                  nperms.act = nperms
            }
            return(list(perms = perms, all.perms.flag =
            all.perms.flag, nperms.act = nperms.act))
      }


##estimate pi0s
pi <- function(testS.p, testS, m){
            qq <- quantile(testS.p, c(0.25, 0.75))
            pi0h <- sum(testS$tt > qq[1] & testS$tt < qq[2])/(0.5
                  * length(testS$tt))

            npos <- sum(testS$tt >= 0)    # number of genes with
                                    #positive test statistic
            nneg <- sum(testS$tt < 0)     # number of genes with
                                    #negative test statistic

            pi0hpos <- (pi0h*m/2)/npos    # estimate of proportion
            #of EE genes with positive test statistics
            pi0hneg <- (pi0h*m/2)/nneg    # estimate of proportion
            #of EE genes with negative test statistics

            return(list(pi0h = pi0h, pi0hpos = pi0hpos, pi0hneg =
            pi0hneg))

      }


##estimate cutup, cutdown, number of significant positive and #negative genes
cut.updn.nsig <- function(testS, deli, tt.bar) {

            tag <- order(testS$tt)
            res.mat <- data.frame(tt = testS$tt[tag], evo =
                        tt.bar, dif = testS$tt[tag] - tt.bar)
            res.up <- res.mat[res.mat$evo > 0, ]
            res.lo <- res.mat[res.mat$evo < 0, ]

            cutup <- rep(1e+10, length(deli))
            cutlow <- rep(-1e+10, length(deli))
            nsig.up <- nsig.lo <- rep(0, length(deli))
            if (nrow(res.up) > 0) {
```

```
                  res.up <- data.frame(dif = res.up$dif, tt =
                              res.up$tt, num = nrow(res.up):1)
                  ## get the upper part
                  j <- 1
                  ii <- 1
                  while (j <= nrow(res.up) & ii <= length(deli)) {
                        if (res.up$dif[j] > deli[ii]) {
                              cutup[ii] <- res.up$tt[j]
                              nsig.up[ii] <- res.up$num[j]
                              ii <- ii + 1
                        }
                        else {
                              j <- j + 1
                        }
                  }
            }
            if (nrow(res.lo) > 0) {
                  res.lo <- data.frame(dif = res.lo$dif, tt =
                              res.lo$tt, num = 1:nrow(res.lo))
                  ## get the lower part
                  j <- nrow(res.lo)
                  ii <- 1
                  while (j >= 1 & ii <= length(deli)) {
                        if (res.lo$dif[j] < -deli[ii]) {
                              cutlow[ii] <- res.lo$tt[j]
                              nsig.lo[ii] <- res.lo$num[j]
                              ii <- ii + 1
                        }
                        else {
                              j <- j - 1
                        }
                  }
            }
            nsig <- nsig.up + nsig.lo
            return(list(cutup = cutup, cutlow = cutlow, nsig =
      nsig, nsig.up = nsig.up, nsig.lo = nsig.lo))
}


##estimate the number of falsely called genes
nfalse <- function(testS.p, cpdn) {
      nfc.up <- matrix(NA, ncol = length(cpdn$cutup), nrow =
      ncol(testS.p))
      nfc.low <- matrix(NA, ncol = length(cpdn$cutlow), nrow =
      ncol(testS.p))

      cutup.rank <- rank(cpdn$cutup, ties.method = "min")
      cutlow.rank <- rank(-cpdn$cutlow, ties.method = "min")

      for (jj in 1:ncol(testS.p)) {

            keep.up <- keep.dn <- testS.p[, jj]

            nfc.up[jj, ] <- length(keep.up) - (rank(c(cpdn$cutup,
                              keep.up), ties.method =
                              "min")[1:length(cpdn$cutup)]
                        - cutup.rank)
```

```
            nfc.low[jj, ] <- length(keep.dn) - (rank(c(-
                        cpdn$cutlow, -keep.dn),ties.method
            = "min")[1:length(cpdn$cutlow)] -
      cutlow.rank)
      }

      nfc <- nfc.up + nfc.low
      return(list(nfc = nfc, nfc.up = nfc.up, nfc.low = nfc.low))
}


################################################################

# Proposed Method I and II

x <- data   # data set
m <- dim(x)[1]    # total number of genes
y <- c(rep(1, dim(x)[2]/2), rep(2, dim(x)[2]/2))      # indicator
                              #for a two class unpaired data

d <- seq.depth(x)       # sequencing depth
xresamp <- resample(x,d)      # resample data


testS <- wilcoxon.unpaired.seq.func(xresamp, y)       # test
                                                      #statistic

perm <- getperms(y,100)       # permutation
b <- perm$nperms.act          # actual number of permutations


permsy <- matrix(y[perm$perms], ncol = length(y))     # indicator
                                    #for permutations based on y
nresamp.perm <- 20            # number of resamples


testS.p <- matrix(0, nrow = nrow(x), ncol = dim(perm$perms)[1])   # permuted
test statistics
for(h in 1:dim(perm$perms)[1]){
      xresamp.p <- xresamp[, , 1:nresamp.perm]
      y.p <- permsy[h, ]
      testS.p[, h] <- wilcoxon.unpaired.seq.func(xresamp.p,
      y.p)$tt     # permuted test statistics

cat("perm = ", 0 + h, "\n")
}


# permuted ordered test statistics
or.testS.p <- apply(testS.p, 2, function(x) -1*sort(-1*x))
or.testS.p <- t(apply(or.testS.p, 1, sort))


# expected ordered statistics
tt.bar <- apply(or.testS.p, 1, mean)
tt.bar <- tt.bar[length(tt.bar):1]
```

```
or.tt <- testS$ordered.tt           # ordered test statistic


# estimate for proposed pi0s
pis <- pi(testS.p, testS, m)


# delta values
deli <- seq(0.01, 1, 0.001)


# estimate cutup, cutdown, number of significant positive(+) and #negative(-)
genes for all delta values
cpdn <- cut.updn.nsig(testS, deli, tt.bar)


# estimate the number of falsely called genes (+/-) for all delta values
nfcb <- nfalse(testS.p, cpdn)


# estimate the median number of falsely called genes (+/-) for #all delta
values
med.nfc.up <- apply(nfcb$nfc.up, 2, median)     # number of falsely
                                    #called positive genes
med.nfc.dn <- apply(nfcb$nfc.low, 2, median)    # number of falsely
                                    #called negative genes


### FDR ESTIMATION ###
### PROPOSED METHOD I ###
p.fdr1 <- ((pis$pi0hpos * med.nfc.up) +
      (pis$pi0hneg*med.nfc.dn)) / (pmax(cpdn$nsig, 1))

### PROPOSED METHOD II ###
# FDR for genes with positive test statistics
fdr2.pos <- (pis$pi0hpos * med.nfc.up) / (pmax(cpdn$nsig.up,1))

# FDR for genes with negative test statistics
fdr2.neg <- (pis$pi0hneg * med.nfc.dn) / (pmax(cpdn$nsig.lo,1))
```