RNAseqPS: A Web Tool for Estimating Sample Size and Power for RNAseq Experiment

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ABSTRACT: Sample size and power determination is the first step in the experimental design of a successful study. Sample size and power calculation is required for applications for National Institutes of Health (NIH) funding. Sample size and power calculation is well established for traditional biological studies such as mouse model, genome wide association study (GWAS), and microarray studies. Recent developments in high-throughput sequencing technology have allowed RNAseq to replace microarray as the technology of choice for high-throughput gene expression profiling. However, the sample size and power analysis of RNAseq technology is an underdeveloped area. Here, we present RNAseqPS, an advanced online RNAseq power and sample size calculation tool based on the Poisson and negative binomial distributions. RNAseqPS was built using the Shiny package in R. It provides an interactive graphical user interface that allows the users to easily conduct sample size and power analysis for RNAseq experimental design. RNAseqPS can be accessed directly at http://cqs.mc.vanderbilt.edu/shiny/RNAseqPS/.

KEYWORDS: RNAseq, power analysis, sample size calculation, experiment design

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Introduction

Gene expression profiling has been an important component of biomedical research. Gene expression is the appearance of a characteristic or effect in the phenotype that can be attributed to a certain gene. For over a decade, microarray technology was the dominating technology for high-throughput gene expression profiling until the introduction of RNAseq technology. RNAseq technology is a form of next-generation sequencing (NGS) technology, sometimes referred to as high-throughput sequencing technology. Since its introduction, NGS technology has revolutionized genetic and biomedical research. RNAseq is the use of NGS technology to sequence cDNA (reversed transcribed from RNA) in order to obtain information about RNA. Compared to microarray technology, RNAseq technology offers several obvious advantages. First, RNAseq allows the detection of all isoforms of a gene, even novel ones. Microarray, on the other hand, relies purely on previous knowledge regarding genes to design probes for detection, thus it cannot be used for novel detection. Second, the resolution of microarray usually stays at the gene and exon level, but the resolution of RNAseq can reach the level of a single nucleotide, which allows detection of single nucleotide variance and structural variants, such as small insertions, deletions, alternative splicing, and gene fusion. Although the cost of RNAseq has become comparable to microarray, researchers have unanimously agreed that RNAseq has replaced microarray as the go technology of high-throughput gene expression profiling.1–4
Although RNAseq technology has introduced exciting opportunities to biomedical researchers, it has also created several challenges for analysts. For example, the data storage cost is significantly higher compared to microarray. And the lack of consensus on the best statistical method for detecting differentially expressed genes\textsuperscript{6} is really noticeable. Another easily overlooked area is sample size and power calculation for RNAseq-based experiment designs. Experiment design is a pivotal step and the first step of any successful study, and one of the most important questions to address during experiment design is what sample size is needed to achieve the desired statistical power within the financial budget. Sample size and power analyses are required for most National Institutes of Health (NIH) funding applications. For microarray-based studies, sample size and power calculation has been well established.\textsuperscript{6-9} The sample size and power for microarray-based studies are relatively easy to compute, because gene expression of microarray data follows a normal distribution. However, RNAseq data are count based, which is usually modeled as a Poisson\textsuperscript{9,10} or negative binomial distribution.\textsuperscript{11,12} Several studies have attempted to produce methods to estimate sample size and power based on Poisson and negative binomial distributions for RNAseq-based experiment design. For example, Fang and Cui\textsuperscript{13} derived a sample size calculation formula based on the Wald test for a single-gene differential expression test. Busby et al.\textsuperscript{14} introduced Scotty, a web tool for computed sample size using a \( t \)-test for RNAseq data based on a Poisson-lognormal distribution. Hart et al.\textsuperscript{15} introduced RNAseqPower, a method for sample size calculation using the score test based on a negative binomial distribution. R code and the excel worksheet for this method were provided by the authors. In reality, RNAseq experiment is capable of detecting thousands of genes, and those genes are tested for differential expression simultaneously. In such cases, the correction of error rates for multiple comparisons is required. However, both Scotty and RNAseqPower fail to discuss how to calculate sample size under multiple comparison testing. To address this issue, Li et al.\textsuperscript{16} derived a sample size calculation formula based on the Poisson distribution. Later, Li et al.\textsuperscript{17} proposed a sample size calculation method based on the exact test.\textsuperscript{18} Of all the developed methods, only Scotty has a web-based graphical user interface. The other proposed methods require running R code. A convenient, user-friendly RNAseq sample size and power calculation tool is highly desirable.

Here, we present RNAseqPS, a web-based power and sample size calculation tool, based on both the Poisson and negative binomial distributions. RNAseqPS is based on the methods\textsuperscript{16,17} developed by Li et al. RNAseqPS distinguishes itself from other RNAseq sample size and power calculation methods in several aspects. First, the graphical user interface of RNAseqPS is highly interactive and intuitive. It offers several different sample size and power calculation methods within in one interface. Also, it does not require the skill of running R code, a person without any previous experience in R can operate it with ease. There are other features of RNAseqPS which are discussed in detail in the Method section.

**Method**

For the Poisson distribution, we considered six different methods: (1) Wald test; (2) score test; (3) likelihood ratio test; (4) log transformation of Wald statistic; (5) log transformation of score statistic; and (6) transformation of Poisson for calculating sample size under both single- and multiple-gene comparison scenarios. The detail of the implementation of these tests can be found.\textsuperscript{16} Under the Poisson distribution, we assume that mean equals variance. When biological replicates are used, RNAseq data could exhibit variation significantly greater than the mean (over-dispersion). In such scenario, the Poisson distribution cannot model the data properly with over-dispersion. The negative binomial distribution can be used as a natural extension of the Poisson model. For the negative binomial distribution, we provide a method to calculate power for the exact test proposed.\textsuperscript{18} based on a given \( P \) value under the single-gene comparison scenario. The detail of the implementation of this test can be found.\textsuperscript{16} To address the multiple comparison issue, we further incorporated false discovery rate (FDR)\textsuperscript{19} controlling into our methods described.\textsuperscript{16,17} RNAseqPS implements sample size and power calculation from both Poisson and negative binomial distributions based on the methods proposed.\textsuperscript{16,17}

**Results**

RNAseqPS is written using the Shiny package in R. Shiny is a powerful web framework for building interactive web applications using R. It combines the computation power of R with the interactivity of modern web. Applications built using Shiny can automatically react with the input and output parameters of an application. Thus, when new parameters are input into RNAseqPS, sample size or power is automatically recomputed and displayed on the screen. RNAseqPS has a very interactive and dynamic graphical user interface (Fig. 1). Users without any experiences in statistics and programing languages can navigate through it without trouble.

RNAseqPS also has the ability to plot the relationship of any parameter with power. For example, a user can specify sample size as the X-axis and selects a reasonable range for sample size. RNAseqPS can plot relationship of the sample size with power while other parameters stay unchanged. Such plots help users visualize the intrinsic relationship between the parameters and power and can be easily exported as high-resolution figures that can be used for NIH grant funding applications. Several examples of such plots can be seen in Figure 2.

Each computation of sample size or power takes up to 30 seconds to 1 minute of time. To reduce the user wait time, we have implemented a database lookup function with RNAseqPS. The database lookup function works as follows.
Figure 1. Example of the graphical interface of RNAseqPS.

Figure 2. Examples of the power curves produced by RNAseqPS.
To estimate the power of the RNAseq experiment under the Poisson distribution, the following eight parameters are required as input: (1) sample size; (2) expected fold change; (3) average reads per gene; (4) read count ratio between two conditions; (5) FDR threshold; (6) total number of genes; (7) expected number of significant genes and (8) dispersion (only required when using negative binomial distribution). The details of these parameters are explained in Table 1. For each of these parameters, there are certain likely ranges. For example, for expected fold change parameter, the range is usually between absolute value 1 to 10 because the absolute value of the fold change cannot be less than 1 and fold change 10 is a reasonable assumption of the high ceiling. To produce the database, we pre-computed the power and sample size using the parameter values within these most likely ranges for all parameters and stored them in a MySQL database. For each round of input parameters, RNAseqPS first queries the database to see if sample size or power for such set of parameters has already been entered into the database. If the input parameters match the existing parameters in the database, the power or sample size is automatically returned, otherwise power or sample size will be computed on the fly. RNAseqPS allows two ways to input parameters: slide bar and text box. When using slide bar, the input is guaranteed to match an input in the database. Using the text box input, user can input any values as desired. In such cases, the sample size and power will be computed on the fly. By using the look up function, we have instant feedback for most of the common RNAseq experiment designs.

Discussion
The dominance of RNAseq over microarray in gene expression research has been documented by multiple studies.1–4 Even though the price of RNAseq has matched that of microarray, large studies can still be costly. Insufficient sample size may lead to underpowered studies and produce unreliable results. Sample size estimation is a critical issue for any biological studies, including RNAseq-based studies. Better management of the tradeoff between cost and sample size is key to a successful study. Sample size and power analysis for RNAseq is an underdeveloped area when compared with microarray. In comparison, sample size and power is easier to calculate for microarray, because microarray follows a normal distribution, while RNAseq data are count based and follow a Poisson or negative binomial distribution. We have discussed several sample size and power calculation methods developed previously. These methods adequately address the sample size and power calculation needs for an RNAseq experiment design but still have two issues. First, some of them do not adjust for the multiple comparisons problem. Second, a majority of them lack an intuitive graphical user interface.

We address these two issues with RNAseqPS. RNAseqPS addresses the multiple comparisons problem by implementing the methods introduced by Li et al., which incorporate FDR controls. Also, RNAseqPS implements methods based on both the Poisson and negative binomial distributions. Overall, there are seven methods implemented in RNAseqPS. Users can select appropriate methods based on the experiment design. Finally, RNAseqPS offers a highly interactive and user-friendly graphical user interface. This graphical user interface helps users input the parameters with ease and greatly enhances users’ abilities to understand the sample size and power analysis with the help of visualization. Overall, RNAseqPS addresses major issues currently lacking in sample size and power analysis for RNAseq experiment design. It greatly benefits researchers who plan to use RNAseq as the primary technology for their study.

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Table 1. RNAseqPS input parameters.

| PARAMETERS                                      | LOWER BOUND | UPPER BOUND | INTERVAL | NOTE                                                                 |
|------------------------------------------------|-------------|-------------|----------|----------------------------------------------------------------------|
| Desired power                                  | 1           | 500         | 1        | Required when computing power                                        |
| Expected fold change                           | 0.8         | 0.95        | 0.05     | Required when computing sample size. The minimum power should be no less than 80% |
| Average reads per gene                         | 1           | 100         | 10       | This can be computed as R/G, where R is the total number of reads sequenced and G is the total number of genes detected |
| Total number genes                             | 100         | 20000       | 100      | This is usually dependent on the gene transfer format (GTF) file used. A GTF file contains the annotation information regarding genes, and it is required for RNAseq analysis |
| Expected number of differentially expressed genes | 5           | 2000        | 50       | This is the number of genes you expect to see between the two conditions. It is also based on prior knowledge. When prior knowledge is unavailable, a best guess is provided by RNAseqPS |
| Dispersion                                     | 0.1         | 2           | 0.1      | This parameter is used in the negative binomial model                |
Author Contributions
YG wrote the manuscript. YG and SZ programmed the user interface. QS helped with the software programming. CL and YS designed the algorithm for RNAseq power analysis and sample size calculation. All authors reviewed and approved of the final manuscript.

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