

# RnaSeqSampleSize: Sample size estimation based on real RNA-seq data

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## Abstract

In this vignette, we demonstrated the application of *RnaSeqSampleSize* as a sample size estimation tool for RNA-seq data. A user friendly web interface is also provided at <http://cqs.mc.vanderbilt.edu/shiny/RnaSeqSampleSize/> for researchers not familiar with R.

*RnaSeqSampleSize* package provides the following features:

- Estimation of sample size or power by single read count and dispersion;
- Estimation of sample size or power by prior real data;
- Visualization of sample size and power by power curves;
- Optimization by power or sample size matrix;

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# 1 Introduction

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Sample size estimation is the most important issue in the design of RNA sequencing experiments. However, thousands of genes are quantified and tested for differential expression simultaneously in RNA-seq experiments. The false discovery rate for statistic tests should be controlled. At the same time, the thousands of genes have widely distributed read counts and dispersions, which were often estimated by experience or set at the most conservative values in previous sample size estimation methods. As a result, the estimated sample size will be inaccurate or over-estimated.

To solve these issues, we developed a sample size estimation method based on the distributions of gene read counts and dispersions from real data. Datasets from the user's preliminary experiments or the Cancer Genome Atlas (TCGA) can be used as reference. The read counts and their related dispersions will be selected randomly from the reference based on their distributions, and from that, the power and sample size will be estimated and summarized.

# 2 User friendly web interface

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A user friendly web interface for *RnaSeqSampleSize* package is provided at <http://cqs.mc.vanderbilt.edu/shiny/RnaSeqSampleSize/>. Most of the functions in Examples section can be performed in this website.

# 3 Examples

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First we will load the *RnaSeqSampleSize* package.

```
library(RnaSeqSampleSize)
## Loading required package: RnaSeqSampleSizeData
## Loading required package: edgeR
## Loading required package: limma
```

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### 3.1 Estimation of sample size or power by single read count and dispersion

#### 3.1.1 Power estimation

For example, if we are estimating the power of finding significant genes for RNA-seq data with specified sample size, and we have the following parameters:

- Number of samples in each group: 63;
- Minimal fold change between two groups: 2;
- Minimal average read counts: 5;
- Maximal dispersion: 0.5;
- False discovery rate: 0.01;

As a result, the estimated power is 0.8 by `est_power` function. It means that we have 80% probability to find the significant genes with 63 samples in each group.

```
example(est_power)
##
## est_pw> n<-63;rho<-2;lambda0<-5;phi0<-0.5;f<-0.01
##
## est_pw> est_power(n=n, rho=rho, lambda0=lambda0, phi0=phi0,f=f)
## [1] 0.8
```

#### 3.1.2 Sample size estimation

For example, if we are estimating the sample size for RNA-seq data to achieve desired power of finding significant genes, and we have the following parameters:

- Desired power of finding significant genes: 0.8;
- Minimal fold change between two groups: 2;
- Minimal average read counts: 5;
- Maximal dispersion: 0.5;
- False discovery rate: 0.01;

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As a result, the estimated sample size is 63 by `sample_size` function. It means that if we want to have 80% probability to find the significant genes, we need 63 samples in each group.

```
example(sample_size)
##
## smpl_s> power<-0.8;rho<-2;lambda0<-5;phi0<-0.5;f<-0.01
##
## smpl_s> sample_size(power=power, f=f,rho=rho, lambda0=lambda0, phi0=phi0)
## [1] 63
```

## 3.2 Estimation of sample size or power by reference data

### 3.2.1 Power estimation with datasets in RnaSeqSampleSizeData package

*RnaSeqSampleSizeData* package contains the read counts and dispersion distribution from some real datasets and can be used as prior data for sample size or power estimation. They can be called with following names:

```
## [1] "TCGA_BLCA" "TCGA_BRCA" "TCGA_CESC" "TCGA_COAD" "TCGA_HNSC" "TCGA_KIRC"
## [7] "TCGA_LGG" "TCGA_LUAD" "TCGA_LUSC" "TCGA_PRAD" "TCGA_READ" "TCGA_THCA"
## [13] "TCGA_UCEC"
```

For example, if we are estimating the power of finding significant genes for RNA-seq data with specified sample size, and we have the following parameters:

- Number of samples in each group: 65;
- Minimal fold change between two groups: 2;
- Prior data: TCGA READ data, stored in *RnaSeqSampleSizeData* package, can be used with name TCGA\_READ;
- False discovery rate: 0.01;

Here we demonstrated the power estimation by prior data in three different situations.

- If we are interested in all genes, we can use `repNumber` parameter to specify random number of genes to perform power estimation;

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```
est_power_distribution(n=65, f=0.01, rho=2,
                      distributionObject="TCGA_READ", repNumber=5)

## [1] 0.9176479
```

Please note here the parameter repNumber was very small (5) to make the example code faster. We suggest repNumber should be at least set as 100 in real analysis.

- If we are only interested in a list of genes, we can use selectedGenes parameter to specify the list of genes to perform power estimation;

```
#Power estimation based on some interested genes.
#We use storeProcess=TRUE to return the details for all selected genes.
selectedGenes<-names(TCGA_READ$pseudo.counts.mean)[c(1,3,5,7,9,12:30)]
powerDistribution<-est_power_distribution(n=65, f=0.01, rho=2,
                                         distributionObject="TCGA_READ",
                                         selectedGenes=selectedGenes,
                                         storeProcess=TRUE)

str(powerDistribution)

## List of 3
## $ power      : num [1:24] 0.8161 0.0547 0.9781 0.9785 0.979 ...
## $ count      : Named num [1:24] 43 36 2000 260 1950 ...
## .. attr(*, "names")= chr [1:24] "A1BG" "A2BP1" "A2M" "A4GALT" ...
## $ dispersion: num [1:24] 0.6 2.3 0.4 0.5 0.1 1.3 0.4 0.1 0.4 0.1 ...

mean(powerDistribution$power)

## [1] 0.8098202
```

- If we are only interested a specified pathway, we can use pathway and species parameters to specify the genes in a pathway to perform power estimation.

```
powerDistribution<-est_power_distribution(n=65, f=0.01, rho=2,
                                         distributionObject="TCGA_READ", pathway="00010",
                                         minAveCount=1, storeProcess=TRUE)

mean(powerDistribution$power)

## [1] 0.7683759
```

As a result, we use `est_power_distribution` function and find the estimated power is 0.91 for random genes, 0.81 for specified gene list, and 0.77 for genes in Glycolysis and Gluconeogenesis (pathway 00010) pathway.

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### 3.2.2 Sample size estimation with datasets in RnaSeqSampleSize-Data package

For example, if we are estimating the sample size for RNA-seq data to achieve desired power of finding significant genes, and we have the following parameters:

- Desired power of finding significant genes: 0.8;
- Minimal fold change between two groups: 2;
- Prior data: TCGA READ data, stored in *RnaSeqSampleSizeData* package, can be used with name TCGA\_READ;
- False discovery rate (FDR): 0.01;

As a result, we use *sample\_size\_distribution* function and find the estimated sample size is 41 for random genes.

```
sample_size_distribution(power=0.8, f=0.01, distributionObject="TCGA_READ",
                        repNumber=5, showMessage=TRUE)

## [1] "x= 1  f(x)= -0.8"
## [1] "x= 33  f(x)= -0.0684782232439268"
## [1] "x= 65  f(x)= 0.113364108578701"
## [1] "x= 49  f(x)= 0.0526440033463642"
## [1] "x= 41  f(x)= 0.00435574028575225"
## [1] "x= 37  f(x)= -0.0281551683359901"
## [1] "x= 39  f(x)= -0.010984381626905"
## [1] "x= 40  f(x)= -0.00308269966285457"
## [1] 41
```

Please note here the parameter `repNumber` was very small (5) to make the example code faster. We suggest `repNumber` should be at least set as 100 in real analysis.

### 3.2.3 Sample size or power estimation with user's prior dataset

For example, if the user has a RNA-seq data with 10000 genes and 10 samples as prior dataset:

```
# Generate a 10000*10 RNA-seq data as prior dataset
set.seed(123)
dataMatrix <- matrix(sample(0:3000, 1e+05, replace = TRUE), nrow = 10000, ncol = 10)
colnames(dataMatrix) <- c(paste0("Control", 1:5), paste0("Treatment", 1:5))
row.names(dataMatrix) <- paste0("gene", 1:10000)
```

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```
head(dataMatrix)

##          Control1 Control2 Control3 Control4 Control5 Treatment1 Treatment2
## gene1          863     932    2974     263     604         639        1270
## gene2         2365     973     906    2339    2935        1331        2357
## gene3         1227    2611    1301     843    1238         384        1806
## gene4         2649     986     481    1649    1426        1434         245
## gene5         2822     377    2469     584      53        2157         885
## gene6          136    1069     624    1018    1104        2718        1016
##          Treatment3 Treatment4 Treatment5
## gene1           561         2056         2504
## gene2           503         1883         2146
## gene3          1922         2294         1235
## gene4          2888         2617         2889
## gene5          1991          917         1676
## gene6           919         2343         1257
```

Then we are estimating the power of finding significant genes for RNA-seq data with specified sample size, and we have the following parameters:

- Number of samples in each group: 65;
- Minimal fold change between two groups: 2;
- Prior data: User's prior dataset with 10000 genes and 10 samples;
- False discovery rate: 0.01;

We will use `est_count_dispersion` to estimate the gene read count and dispersion distribution of user's prior dataset. And then `est_power_distribution` function will be used to estimate power.

```
#Estimate the gene read count and dispersion distribution
dataMatrixDistribution<-est_count_dispersion(dataMatrix,
                                             group=c(rep(0,5),rep(1,5)))

## Disp = 0.59727 , BCV = 0.7728

#Power estimation by read count and dispersion distribution
est_power_distribution(n=65,f=0.01,rho=2,
                      distributionObject=dataMatrixDistribution,repNumber=5)

## [1] 0.9131214
```

As a result, we can find the estimated power is 0.91. Please note here the parameter `repNumber` was very small (5) to make the example code faster. We suggest `repNumber` should be at least set as 100 in real analysis.

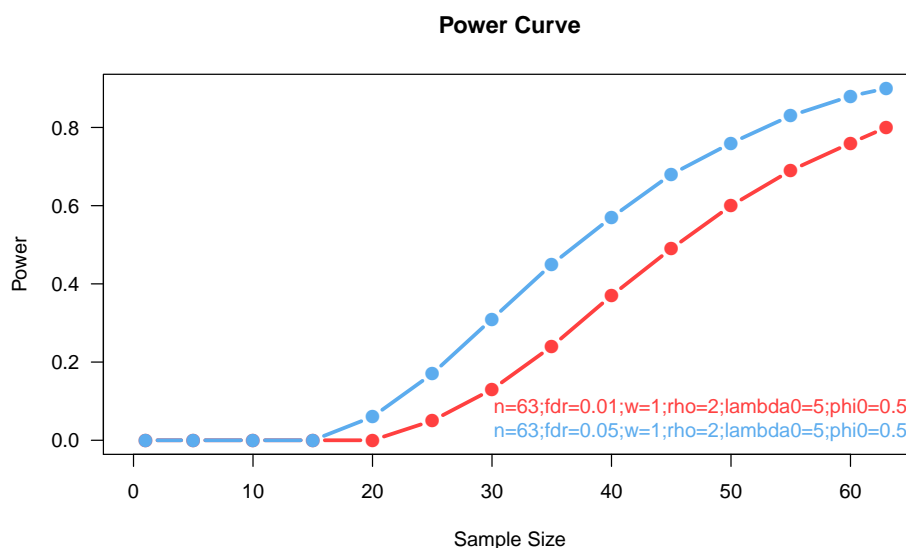


### 3.3 Power curve visualization

For example, if we are going to compare the power of finding significant genes for different false discovery rate, and we have the following parameters:

- Number of samples in each group: 63;
- Minimal fold change between two groups: 2;
- Minimal average read counts: 5;
- Maximal dispersion: 0.5;
- False discovery rate: 0.01 and 0.05;

```
result1 <- est_power_curve(n = 63, f = 0.01, rho = 2, lambda0 = 5, phi0 = 0.5)
result2 <- est_power_curve(n = 63, f = 0.05, rho = 2, lambda0 = 5, phi0 = 0.5)
plot_power_curve(list(result1, result2))
```



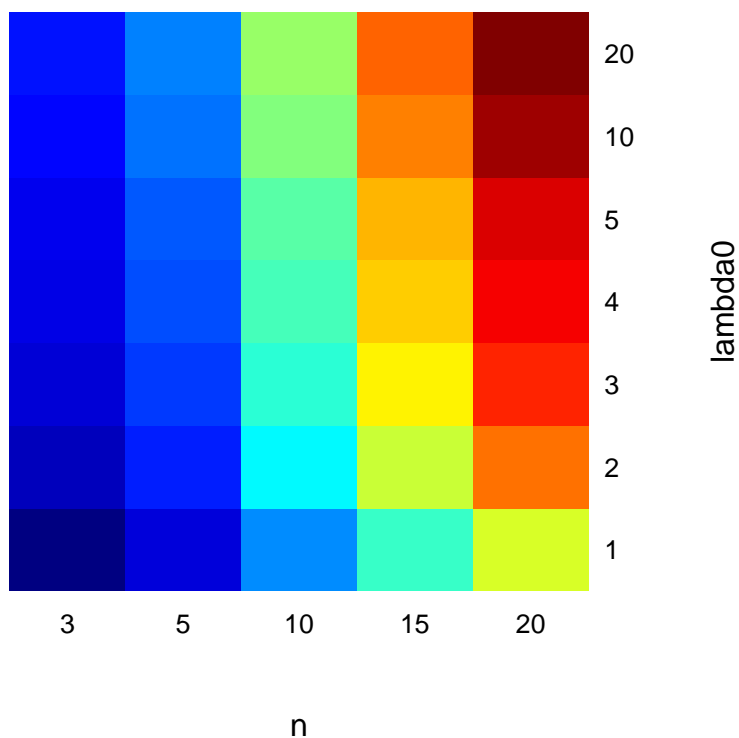
As a result, the relation between power and sample size can be estimated by `est_power_curve` function and the power curves can be generated by `plot_power_curve` function.

### 3.4 Optimization by power or sample size matrix

For example, if the budget is limited, we need to balance the number of replications and sequence depth. We can use the `optimize_parameter` function to find the relation between sample size, read counts, and estimated power. And then the optimized parameters can be determined.

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```
result<-optimize_parameter(fun=est_power,opt1="n",  
                           opt2="lambda0",opt1Value=c(3,5,10,15,20),  
                           opt2Value=c(1:5,10,20))
```



As a result, the estimated power distribution indicates that the number of replications plays a more significant role in determining the power than the number of read counts.