metaSeq: Meta-analysis of RNA-seq count data

Koki Tsuyuzaki[1], and Itoshi Nikaido[2].

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[1]Department of Medical and Life Science, Tokyo University of Science.
[2]Bioinformatics Research Unit, Advanced Center for Computing and Communication, RIKEN.

k.t.the-answer@hotmail.co.jp

Contents

1 Introduction

This document provides the way to perform meta-analysis of RNA-seq data using *metaSeq* package. Meta-analysis is a attempt to integrate multiple data in different studies and retrieve much reliable and reproducible result. In our package, the probability of one-sided *NOISeq* [1] is applied in each study. This is because the numbers of reads are often different depending on its study and *NOISeq* is robust method against its difference (see the next section).

2 RSE: Read-Size Effect

In many cases, the number of reads are depend on study. For example, here we prepared multiple RNA-Seq count data designed as Breast Cancer cell lines vs Normal cells measured in 4 different studies (this data is also accessible by data(BreastCancer)).

ID in this vignette	Accession (SRA / ERA Accession)	Experimental Design
StudyA	SRP008746	Breast Cancer (n=3) vs Normal (n=2)
StudyB	SRP006726	Breast Cancer (n=1) vs Normal (n=1)
StudyC	SRP005601	Breast Cancer (n=7) vs Normal (n=1)
StudyD	ERP000992	Breast Cancer (n=2) vs Normal (n=1)

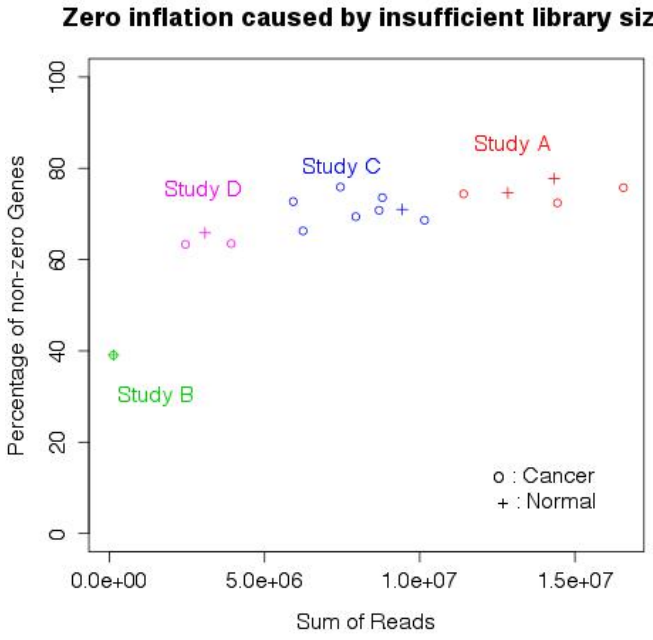

Figure 1: Difference of the number of reads

As shown in the figure 1, the number of reads in StudyA, B, C, and D are relatively different. Generally, statistical test is influenced by the number of reads; the more the number of reads is large, the more the statistical tests are tend to be significant (see the next section). Therefore, in meta-analysis of RNA-seq data, data may be suffered from this bias. Here we call this bias as RSE (Read Size Effect).

3 Robustness against RSE

In the point of view of robustness against RSE, we evaluated five widely used method in RNA-seq; *DESeq* [2], *edgeR* [3], *baySeq* [4], and *NOISeq* [1]. Here we used only StudyA data. All counts in the matrix are repeatedly down-sampled in accordance with distributions of binomial (the probability equals 0.5). 1 (original), 1/2, 1/4, 1/8, 1/16, and 1/32-fold data are prepared as low read size situation. In each read size, four methods are conducted (figure 2.A, this data is also accessible by data(StudyA) and data(pvals)), then we focussed on how top500 genes of original data in order of significance will change its members, influenced by low read size (figure 2.B).

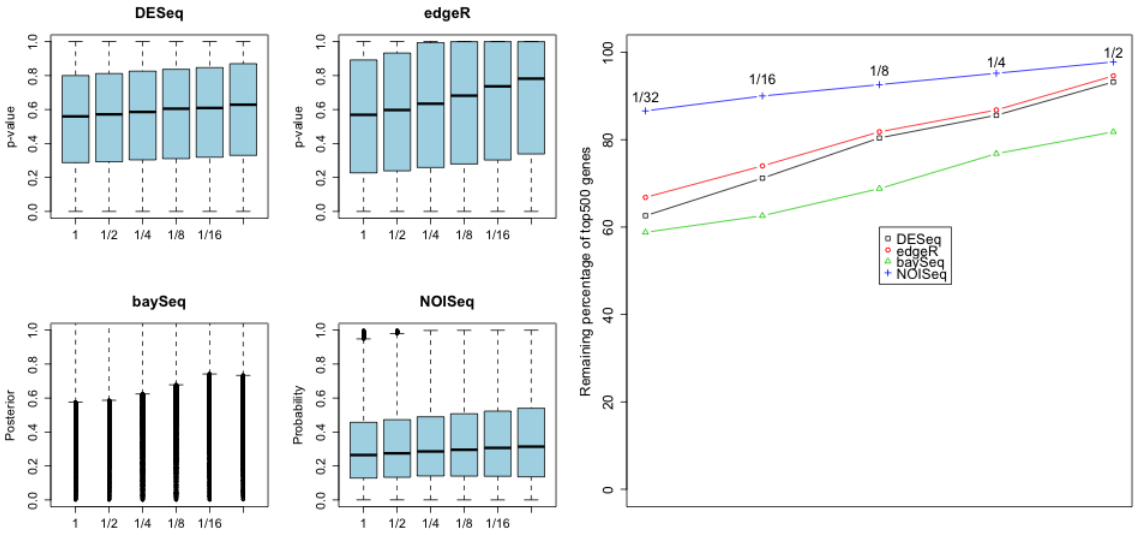

Figure 2: A(left): RSE in each RNA-Seq method, B(right): Top 500 genes in order of significance

Ideal method will returns same result regardless of read size, because same data was used. As shown in figure 2, *NOISeq* is not almost affected by the number of reads and robustlly detects same genes as DEGs. Therefore, we concluded that *NOISeq* is suitable method at least in the point of view of meta-analysis. Note that probability of *NOISeq* is not equal to p-value; it is the probability that a gene is differentially expressed [1]. Our package integrates its probability by Fisher's method [5] or Stouffer's method (inverse normal method) [6]. In regard to Stouffer's method, weighting by the number of replicates (sample size) is used.

4 Getting started

At first, install and load the *metaSeq* and *snow*.

```
> library("metaSeq")
> library("snow")
```

The RNA-seq expression data in breast cancer cell lines and normal cells is prepared. The data is measured from 4 different studies.The data is stored as a matrix (23368 rows $\times$ 18 columns).

```
> data(BreastCancer)
```

We need to prepare two vectors. First vector is for indicating the experimental condition (e.g., 1: Cancer, 2: Normal) and second one is for indicating the source of data (e.g., A: StudyA, B: StudyB, C: StudyC, D: StudyD).

```
> flag1 <- c(1,1,1,0,0, 1,0, 1,1,1,1,1,1,1,0, 1,1,0)
> flag2 <- c("A","A","A","A","A", "B","B", "C","C","C","C","C","C","C","C", "D","D","D")
```

Then, we use meta.readData to create R object for meta.oneside.noiseq.

```
> cds <- meta.readData(data = BreastCancer, factor = flag1, studies = flag2)
```

oneside.noiseq is performed in each studies and each probabilities are summalized as member of list object.

```
> ## This is very time consuming step.
> # cl <- makeCluster(4, "SOCK")
> # result <- meta.oneside.noiseq(cds, k = 0.5, norm = "tmm", replicates = "biological",
> #  factor = flag1, conditions = c(1, 0), studies = flag2, cl = cl)
> # stopCluster(cl)
>
> ## Please load pre-calculated result (Result.Meta)
> ## by data function instead of scripts above.
> data(Result.Meta)
> result <- Result.Meta
```

Fisher's method and Stouffer's method can be applied to the result of meta.oneside.noiseq.

```
> F <- Fisher.test(result)
> S <- Stouffer.test(result)
```

These outputs are summalized as list whose length is 3. First member is the probability which means a gene is upper-regulated genes, and Second member is lower-regulated genes. Weight in each study is also saved as its third member (weight is used only by Stouffer's method).

```
> head(F$Upper)
```

```
1/2-SBSRNA4        A1BG    A1BG-AS1        A1CF       A2LD1
  0.3842542   0.5316118   0.5325544          NA   0.1358559
        A2M
  0.2252807

> head(F$Lower)

1/2-SBSRNA4        A1BG    A1BG-AS1        A1CF       A2LD1
  0.8420357   0.6078896   0.4047202          NA   0.3661371
        A2M
  0.6197968

> F$Weight

Study 1 Study 2 Study 3 Study 4
      5       2       8       3

> head(S$Upper)

1/2-SBSRNA4        A1BG    A1BG-AS1        A1CF       A2LD1
  0.3709297   0.2663748   0.2711745          NA   0.2957139
        A2M
  0.2996707

> head(S$Lower)

1/2-SBSRNA4        A1BG    A1BG-AS1        A1CF       A2LD1
  0.6290703   0.7336252   0.7288255          NA   0.7042861
        A2M
  0.7003293

> S$Weight

Study 1 Study 2 Study 3 Study 4
      5       2       8       3
```

5 Meta-analysis by non-NOISeq method

For some reason, we may want to use non-NOISeq method like *DESeq*, *edgeR*, or even cuffdiff [7]. We prepared other.oneside.noiseq as optional function for such methods. Returned object can be directlly applied for Fisher.test and Stouffer.test.

```
> ## Assume this matrix as one-sided p-values
> ## generated by non-NOISeq method (e.g., cuffdiff)
> upper <- matrix(runif(300), ncol=3, nrow=100)
> lower <- 1 - upper
> rownames(upper) <- paste0("Gene", 1:100)
```

```
> rownames(lower) <- paste0("Gene", 1:100)
> weight <- c(3,6,8)
> ## other.oneside.pvalues function return a matrix
> ## which can input Fisher.test or Stouffer.test
> result <- other.oneside.pvalues(upper, lower, weight)
> ## Fisher's method (without weighting)
> F <- Fisher.test(result)
> str(F)

List of 3
 $ Upper : Named num [1:100] 0.2689 0.7954 0.361 0.6798 0.0563 ...
  ..- attr(*, "names")= chr [1:100] "Gene1" "Gene2" "Gene3" "Gene4" ...
 $ Lower : Named num [1:100] 0.652 0.147 0.606 0.584 0.97 ...
  ..- attr(*, "names")= chr [1:100] "Gene1" "Gene2" "Gene3" "Gene4" ...
 $ Weight: Named num [1:3] 3 6 8
  ..- attr(*, "names")= chr [1:3] "Exp 1" "Exp 2" "Exp 3"

> F

$Upper
     Gene1      Gene2      Gene3      Gene4      Gene5      Gene6
0.26887544 0.79539709 0.36104357 0.67981904 0.05630875 0.82815743
     Gene7      Gene8      Gene9     Gene10     Gene11     Gene12
0.79533495 0.55382732 0.21703959 0.46588908 0.15349836 0.27396489
    Gene13     Gene14     Gene15     Gene16     Gene17     Gene18
0.39098451 0.21673232 0.39577063 0.22993103 0.68103680 0.40779439
    Gene19     Gene20     Gene21     Gene22     Gene23     Gene24
0.65653452 0.54909973 0.43785146 0.56620293 0.04103333 0.77685804
    Gene25     Gene26     Gene27     Gene28     Gene29     Gene30
0.40403225 0.86068346 0.32396153 0.36745680 0.73127190 0.75932992
    Gene31     Gene32     Gene33     Gene34     Gene35     Gene36
0.45392988 0.56336136 0.05003923 0.30386104 0.85535751 0.57161305
    Gene37     Gene38     Gene39     Gene40     Gene41     Gene42
0.10057188 0.34761241 0.45937419 0.33926033 0.93159012 0.85095826
    Gene43     Gene44     Gene45     Gene46     Gene47     Gene48
0.21624892 0.29152807 0.83852383 0.83659417 0.45638506 0.12427522
    Gene49     Gene50     Gene51     Gene52     Gene53     Gene54
0.08333039 0.36126924 0.08134459 0.60074658 0.66907227 0.74178398
    Gene55     Gene56     Gene57     Gene58     Gene59     Gene60
0.83135209 0.72240983 0.58649083 0.46409292 0.29577362 0.15142499
    Gene61     Gene62     Gene63     Gene64     Gene65     Gene66
0.29691563 0.03566004 0.27928016 0.10460455 0.33510078 0.28685043
    Gene67     Gene68     Gene69     Gene70     Gene71     Gene72
0.43248838 0.85950235 0.21733554 0.92370472 0.39227056 0.51664371
    Gene73     Gene74     Gene75     Gene76     Gene77     Gene78
0.78890398 0.49982276 0.19614476 0.69264709 0.09286959 0.80558715
```

```
    Gene79     Gene80     Gene81     Gene82     Gene83     Gene84
0.64543693 0.68563273 0.88759542 0.37649581 0.10647869 0.75005451
    Gene85     Gene86     Gene87     Gene88     Gene89     Gene90
0.11184655 0.71920611 0.09242819 0.20858119 0.47627566 0.94984431
    Gene91     Gene92     Gene93     Gene94     Gene95     Gene96
0.78124740 0.70633272 0.44185256 0.10880750 0.74041350 0.83573005
    Gene97     Gene98     Gene99    Gene100
0.59406789 0.26432207 0.78431898 0.39466776

$Lower
      Gene1       Gene2       Gene3       Gene4       Gene5
0.652413882 0.146501514 0.606131358 0.583686392 0.970398775
      Gene6       Gene7       Gene8       Gene9      Gene10
0.383833923 0.371901383 0.396517272 0.630097959 0.502001556
     Gene11      Gene12      Gene13      Gene14      Gene15
0.657769400 0.596121163 0.655262341 0.940133552 0.596550388
     Gene16      Gene17      Gene18      Gene19      Gene20
0.881225316 0.371145361 0.699595754 0.129714674 0.734718137
     Gene21      Gene22      Gene23      Gene24      Gene25
0.403311827 0.620632192 0.469715168 0.478700011 0.512975131
     Gene26      Gene27      Gene28      Gene29      Gene30
0.111416168 0.878635760 0.570622626 0.178330353 0.448206702
     Gene31      Gene32      Gene33      Gene34      Gene35
0.740550638 0.484900496 0.577012268 0.678935220 0.239536749
     Gene36      Gene37      Gene38      Gene39      Gene40
0.239644653 0.592008374 0.639015089 0.624452038 0.432982805
     Gene41      Gene42      Gene43      Gene44      Gene45
0.005413383 0.338316468 0.352161821 0.852883888 0.245333232
     Gene46      Gene47      Gene48      Gene49      Gene50
0.213024645 0.678752560 0.958092437 0.901033505 0.087289165
     Gene51      Gene52      Gene53      Gene54      Gene55
0.933160978 0.675950433 0.283400160 0.030181120 0.237060195
     Gene56      Gene57      Gene58      Gene59      Gene60
0.440804184 0.631823149 0.367323141 0.389547653 0.924748936
     Gene61      Gene62      Gene63      Gene64      Gene65
0.302949943 0.980232102 0.848895645 0.501601400 0.722554083
     Gene66      Gene67      Gene68      Gene69      Gene70
0.392372034 0.634429095 0.333286720 0.232123514 0.229197237
     Gene71      Gene72      Gene73      Gene74      Gene75
0.823821663 0.305617637 0.381460004 0.719090100 0.901096726
     Gene76      Gene77      Gene78      Gene79      Gene80
0.398397364 0.940038105 0.351058446 0.571450551 0.208109906
     Gene81      Gene82      Gene83      Gene84      Gene85
0.043055097 0.727524598 0.867068540 0.407251559 0.788133895
     Gene86      Gene87      Gene88      Gene89      Gene90
```

```
0.421128012 0.974720009 0.550183129 0.729664104 0.145350901
     Gene91      Gene92      Gene93      Gene94      Gene95
0.333817298 0.483575022 0.630163430 0.977988047 0.276865051
     Gene96      Gene97      Gene98      Gene99     Gene100
0.380463488 0.711097281 0.910599913 0.321765232 0.833695436

$Weight
Exp 1 Exp 2 Exp 3
    3     6     8

> ## Stouffer's method (with weighting by sample-size)
> S <- Stouffer.test(result)
> str(S)

List of 3
 $ Upper : Named num [1:100] 0.5157 0.9336 0.5047 0.6159 0.0481 ...
  ..- attr(*, "names")= chr [1:100] "Gene1" "Gene2" "Gene3" "Gene4" ...
 $ Lower : Named num [1:100] 0.4843 0.0664 0.4953 0.3841 0.9519 ...
  ..- attr(*, "names")= chr [1:100] "Gene1" "Gene2" "Gene3" "Gene4" ...
 $ Weight: Named num [1:3] 3 6 8
  ..- attr(*, "names")= chr [1:3] "Exp 1" "Exp 2" "Exp 3"

> S

$Upper
     Gene1      Gene2      Gene3      Gene4      Gene5      Gene6
0.51566590 0.93360429 0.50465740 0.61594795 0.04809289 0.78095817
     Gene7      Gene8      Gene9     Gene10     Gene11     Gene12
0.73879607 0.66526340 0.15247161 0.55364801 0.47305001 0.16876705
    Gene13     Gene14     Gene15     Gene16     Gene17     Gene18
0.50900267 0.13788049 0.50147844 0.11058371 0.75395398 0.46938711
    Gene19     Gene20     Gene21     Gene22     Gene23     Gene24
0.93011113 0.36975078 0.70782562 0.37963374 0.32689138 0.59348708
    Gene25     Gene26     Gene27     Gene28     Gene29     Gene30
0.65730946 0.93395906 0.19188184 0.55999159 0.76472885 0.68237794
    Gene31     Gene32     Gene33     Gene34     Gene35     Gene36
0.45396734 0.68144710 0.11795119 0.25030442 0.88362585 0.78519020
    Gene37     Gene38     Gene39     Gene40     Gene41     Gene42
0.08082026 0.19222602 0.56405405 0.34578329 0.96950421 0.72584227
    Gene43     Gene44     Gene45     Gene46     Gene47     Gene48
0.73480594 0.15282272 0.71718408 0.83583474 0.40574373 0.12455979
    Gene49     Gene50     Gene51     Gene52     Gene53     Gene54
0.06932575 0.60336704 0.05248166 0.50250789 0.51059388 0.73325549
    Gene55     Gene56     Gene57     Gene58     Gene59     Gene60
0.86519273 0.54103207 0.55896221 0.31096706 0.29559913 0.08256385
    Gene61     Gene62     Gene63     Gene64     Gene65     Gene66
```

```
0.76731010 0.06039796 0.23577510 0.14977388 0.41886799 0.52027545
    Gene67     Gene68     Gene69     Gene70     Gene71     Gene72
0.54837777 0.75699537 0.38404579 0.81633138 0.24351709 0.54614700
    Gene73     Gene74     Gene75     Gene76     Gene77     Gene78
0.61973364 0.47941238 0.18035215 0.55852283 0.12056559 0.75092693
    Gene79     Gene80     Gene81     Gene82     Gene83     Gene84
0.44281398 0.89367247 0.97513270 0.43650262 0.04346274 0.56775677
    Gene85     Gene86     Gene87     Gene88     Gene89     Gene90
0.31173151 0.66785495 0.07147003 0.50816911 0.41174256 0.88276660
    Gene91     Gene92     Gene93     Gene94     Gene95     Gene96
0.77119172 0.50594707 0.33313595 0.06181756 0.70019218 0.75300966
    Gene97     Gene98     Gene99    Gene100
0.45246476 0.14707687 0.74644736 0.30893299

$Lower
     Gene1      Gene2      Gene3      Gene4      Gene5      Gene6
0.48433410 0.06639571 0.49534260 0.38405205 0.95190711 0.21904183
     Gene7      Gene8      Gene9     Gene10     Gene11     Gene12
0.26120393 0.33473660 0.84752839 0.44635199 0.52694999 0.83123295
    Gene13     Gene14     Gene15     Gene16     Gene17     Gene18
0.49099733 0.86211951 0.49852156 0.88941629 0.24604602 0.53061289
    Gene19     Gene20     Gene21     Gene22     Gene23     Gene24
0.06988887 0.63024922 0.29217438 0.62036626 0.67310862 0.40651292
    Gene25     Gene26     Gene27     Gene28     Gene29     Gene30
0.34269054 0.06604094 0.80811816 0.44000841 0.23527115 0.31762206
    Gene31     Gene32     Gene33     Gene34     Gene35     Gene36
0.54603266 0.31855290 0.88204881 0.74969558 0.11637415 0.21480980
    Gene37     Gene38     Gene39     Gene40     Gene41     Gene42
0.91917974 0.80777398 0.43594595 0.65421671 0.03049579 0.27415773
    Gene43     Gene44     Gene45     Gene46     Gene47     Gene48
0.26519406 0.84717728 0.28281592 0.16416526 0.59425627 0.87544021
    Gene49     Gene50     Gene51     Gene52     Gene53     Gene54
0.93067425 0.39663296 0.94751834 0.49749211 0.48940612 0.26674451
    Gene55     Gene56     Gene57     Gene58     Gene59     Gene60
0.13480727 0.45896793 0.44103779 0.68903294 0.70440087 0.91743615
    Gene61     Gene62     Gene63     Gene64     Gene65     Gene66
0.23268990 0.93960204 0.76422490 0.85022612 0.58113201 0.47972455
    Gene67     Gene68     Gene69     Gene70     Gene71     Gene72
0.45162223 0.24300463 0.61595421 0.18366862 0.75648291 0.45385300
    Gene73     Gene74     Gene75     Gene76     Gene77     Gene78
0.38026636 0.52058762 0.81964785 0.44147717 0.87943441 0.24907307
    Gene79     Gene80     Gene81     Gene82     Gene83     Gene84
0.55718602 0.10632753 0.02486730 0.56349738 0.95653726 0.43224323
    Gene85     Gene86     Gene87     Gene88     Gene89     Gene90
0.68826849 0.33214505 0.92852997 0.49183089 0.58825744 0.11723340
```

```
    Gene91     Gene92     Gene93     Gene94     Gene95     Gene96
0.22880828 0.49405293 0.66686405 0.93818244 0.29980782 0.24699034
    Gene97     Gene98     Gene99    Gene100
0.54753524 0.85292313 0.25355264 0.69106701

$Weight
Exp 1 Exp 2 Exp 3
    3     6     8
```

6 Setup

This vignette was built on:

```
> sessionInfo()

R version 3.0.1 (2013-05-16)
Platform: x86_64-apple-darwin10.8.0 (64-bit)

locale:
[1] ja_JP.UTF-8/ja_JP.UTF-8/ja_JP.UTF-8/C/ja_JP.UTF-8/ja_JP.UTF-8

attached base packages:
[1] splines   parallel  stats     graphics  grDevices utils
[7] datasets  methods   base

other attached packages:
[1] metaSeq_0.99.0     snow_0.3-12        NOISeq_2.0.0
[4] Biobase_2.20.1     BiocGenerics_0.6.0

loaded via a namespace (and not attached):
[1] tools_3.0.1
```

References

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