Introduction to RNASeq

Malay (malay@uab.edu)

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1 Introduction

RNASeq is a very vast topic and tons of papers have been and are being written on the topic. The following is just an overview.

Originally the idea was proposed by Mortazavi et al. (2008). Although several modifciation of the original idea have been developed, the basics did not change. In this handout will use the latest in the RNASeq methodology through the use of software called RSEM (Li and Dewey, 2011).

2 Normalization of RNASeq data

People have proposed several methods of normalization of RNASeq data. For a comparison see Dillies et al. (2013).

3 Datasets

Every diffential expression measurment should have biological replicates. For demonstration, we will use only 1 replicate for two biological conditions. But in real life, this should never be used. We will use two small datasets from Illumina Body Map project. These are samples prpared from adrenal gland and brain and only from chromosome 19. You can download the datasets here:

http://cmb.path.uab.edu/training/docs/CB2-201-2015/rnaseq_data.tar.gz

Unzip the file.

4 STAR

STAR is a modern fast aligner for RNASeq data to reference genome.

```
wget https://github.com/alexdobin/STAR/archive/2.5.1b.tar.gz
tar -xvzf 2.5.1b.tar.gz
cd STAR-2.5.1b
make
```

Put the software in your path

```
cd Linux_x86_64_static/
export PATH=$PATH:`pwd`
```

Prepare the referene genome:

```
mkdir hs
STAR --runThreadN 8 --genomeDir hs --runMode genomeGenerate \
   --genomeFastaFiles chr19.fa --sjdbGTFfile human_chr19.gtf
```

Now create the alignment. There is a special option for STAR to create a "transcriptome alignment" that could be fed directly to RSEM.

```
STAR --runThreadN 8 --genomeDir hs --readFilesIn adrenal_R1.fq \
    adrenal_R2.fq --quantMode TranscriptomeSAM
```

5 RSEM

RSEM is a cutting-edge RNASeq analysis package that is an end-to-end solution for differential expression, and simplifies the whole process. It also intriduces a new more robust unit of RNASeq measurement called TPM.

5.1 Installing RSEM

```
wget http://deweylab.biostat.wisc.edu/rsem/src/rsem-1.2.19.tar.gz
tar -xvzf rsem-1.2.19.tar.gz
cd rsem-1.2.19/
make
export PATH=$PATH:`pwd`

# Install ebseq
module load R/R-3.1.2
make ebseq
cd EBSeq/
export PATH=$PATH:`pwd`
```

5.2 Prepare reference

```
rsem-prepare-reference --gtf human_chr19.gtf chr19.fa rsem/chr19
```

5.3 Calculate expression directly from STAR output

```
rsem-calculate-expression --no-bam-output --paired-end \
  --bam Aligned.toTranscriptome.out.bam rsem/chr19 adrenal
```

5.4 Simpler way to estimating expression

```
rsem-prepare-reference --gtf human_chr19.gtf --star --star-path \
  ../STAR-2.5.1b/bin/Linux_x86_64_static -p 8 chr19.fa hs/chr19
rsem-calculate-expression  --paired-end --star --star-path \
  ../STAR-2.5.1b/bin/Linux_x86_64_static/ -p 8 adrenal_R1.fq \
  adrenal_R2.fq hs/chr19 adrenal_rsem
rsem-calculate-expression  --paired-end --star --star-path \
  ../STAR-2.5.1b/bin/Linux_x86_64_static/ -p 8 brain_R1.fq brain_R2.fq \
  hs/chr19 brain_rsem
```

5.5 Differential expression

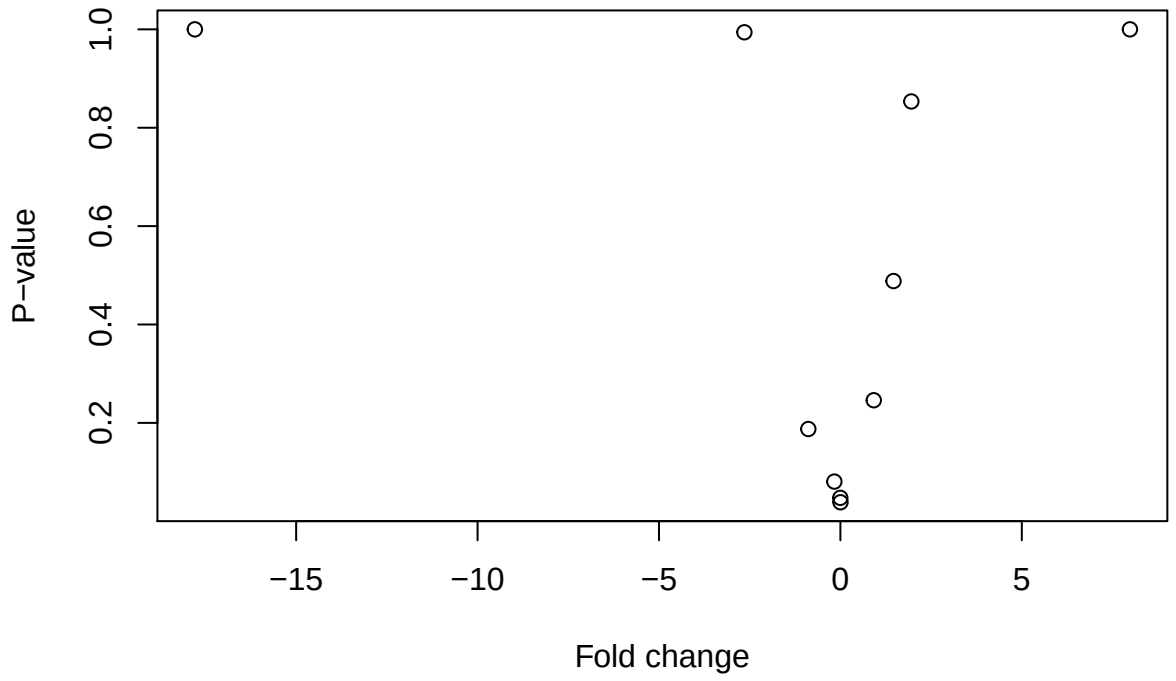
```
rsem-generate-data-matrix adrenal_chr19.genes.results human_chr19.genes.results \
  >diff-brain-adrenal.txt
rsem-run-ebseq diff-brain-adrenal.txt 1,1 expression.results.txt
rsem-control-fdr expression.results.txt 0.05 expression_final.txt
```

And we have our differentially expressed genes.

5.6 Volcano plot

Volcano plot is a good way to show the differentially expressed genes. For that we need the p-value for the differentially expressed genes and the the fold change. Given by "PPEE" and "RealFC" values.

```
data<-read.table("expression.results.txt")
plot(log2(data$RealFC),data$PPDE,xlab="Fold change",ylab="P-value")
```



6 EDGER

```
raw.data <- read.table("../data/pnas_expression.txt",header=T)
head(raw.data)
```

```
##        ensembl_ID lane1 lane2 lane3 lane4 lane5 lane6 lane8  len
## 1 ENSG00000215696     0     0     0     0     0     0     0  330
## 2 ENSG00000215700     0     0     0     0     0     0     0 2370
## 3 ENSG00000215699     0     0     0     0     0     0     0 1842
## 4 ENSG00000215784     0     0     0     0     0     0     0 2393
## 5 ENSG00000212914     0     0     0     0     0     0     0  384
## 6 ENSG00000212042     0     0     0     0     0     0     0   92
```

```
counts <- raw.data[ , -c(1,ncol(raw.data))]
rownames(counts) <- raw.data$ensembl_ID
colnames(counts)  <- paste(c(rep("C_R",4),rep("T_R",3)),c(1:4,1:3),sep="")

library(edgeR)
```

```
## Loading required package: limma
```

```
group <- c(rep("C", 4) , rep("T", 3))
cds <- DGEList( counts , group = group )
cds <- calcNormFactors(cds)
design <- model.matrix(~group)
y <- estimateDisp(cds, design)
fit <- glmQLFit(y,design)
qlf <- glmQLFTest(fit,coef=2)
topTags(qlf)
```

```
## Coefficient:  groupT
##                     logFC    logCPM         F       PValue          FDR
## ENSG00000151503 5.803395  9.708595 2588.8189 2.213389e-37 8.285821e-33
## ENSG00000096060 4.992027  9.941858 2407.1639 8.999503e-37 1.684482e-32
## ENSG00000166451 4.668930  8.840505 1567.1954 3.402099e-33 4.245252e-29
## ENSG00000162772 3.304605  9.736944 1160.1751 1.041278e-30 9.745059e-27
## ENSG00000115648 2.586936 11.468899 1104.3198 2.650584e-30 1.984492e-26
## ENSG00000127954 8.109679  7.210229  964.5570 3.409069e-29 2.126975e-25
## ENSG00000123983 3.638588  8.591116  946.5614 4.859487e-29 2.598784e-25
## ENSG00000113594 4.097420  8.046388  934.3996 6.197792e-29 2.900179e-25
## ENSG00000116133 3.232335  8.785966  883.2447 1.784727e-28 7.423473e-25
## ENSG00000130066 2.596572  9.981348  875.4858 2.106016e-28 7.883871e-25
```

Bibliography

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Li,B. and Dewey,C.N. (2011) RSEM: Accurate transcript quantification from RNA-seq data with or without a reference genome. *BMC Bioinformatics*, **12**, 323.

Mortazavi,A. *et al.* (2008) Mapping and quantifying mammalian transcriptomes by RNA-seq. *Nat. Methods*, **5**, 621–628.