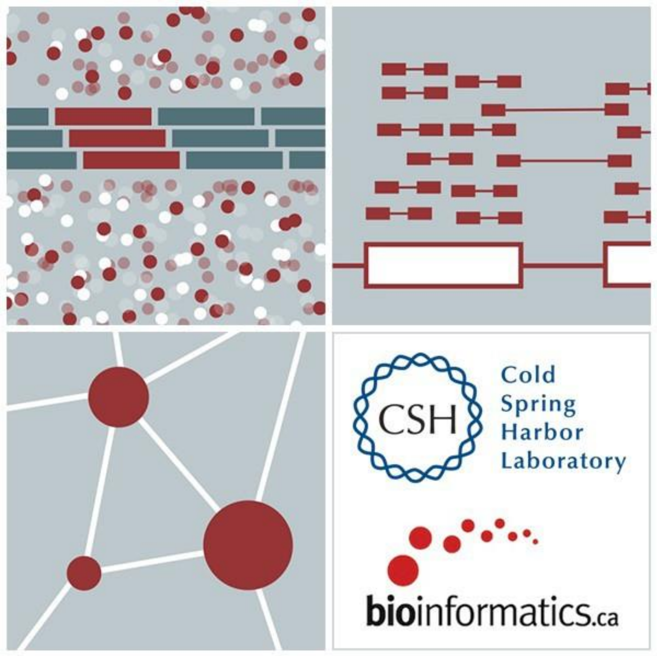
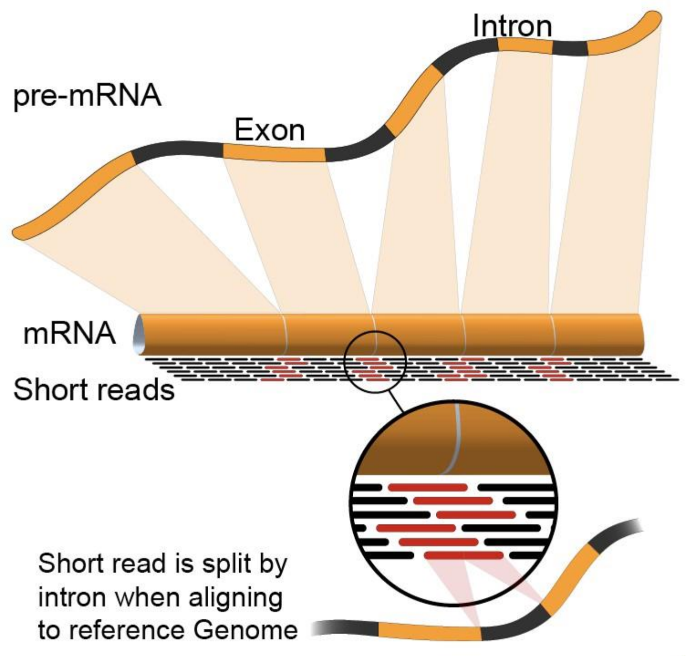
RNA-Seq Module 3
Differential Expression

Mariam Khanfar

Bfx workshop_Dec 11, 2023

Slides adapted from CSHL SeqTec course RNA-seq lecture series by Obi & Malachi Griffith





Washington University in St. Louis
School of Medicine

Learning Objectives of Module 3

- Expression estimation for known genes and transcripts
- FPKM/TPM expression normalized vs. raw counts
- Differential expression methods

Learning Objectives of Module 3

- ~~Expression estimation for known genes and transcripts~~
- ~~FPKM/TPM expression normalized vs. raw counts~~
- Differential expression methods → Ballgown (Stringtie)

To-do

- Open docker desktop app in the background. Then in terminal, type:

  ```
  $ docker pull griffithlab/rnabio:0.0.1
  ```

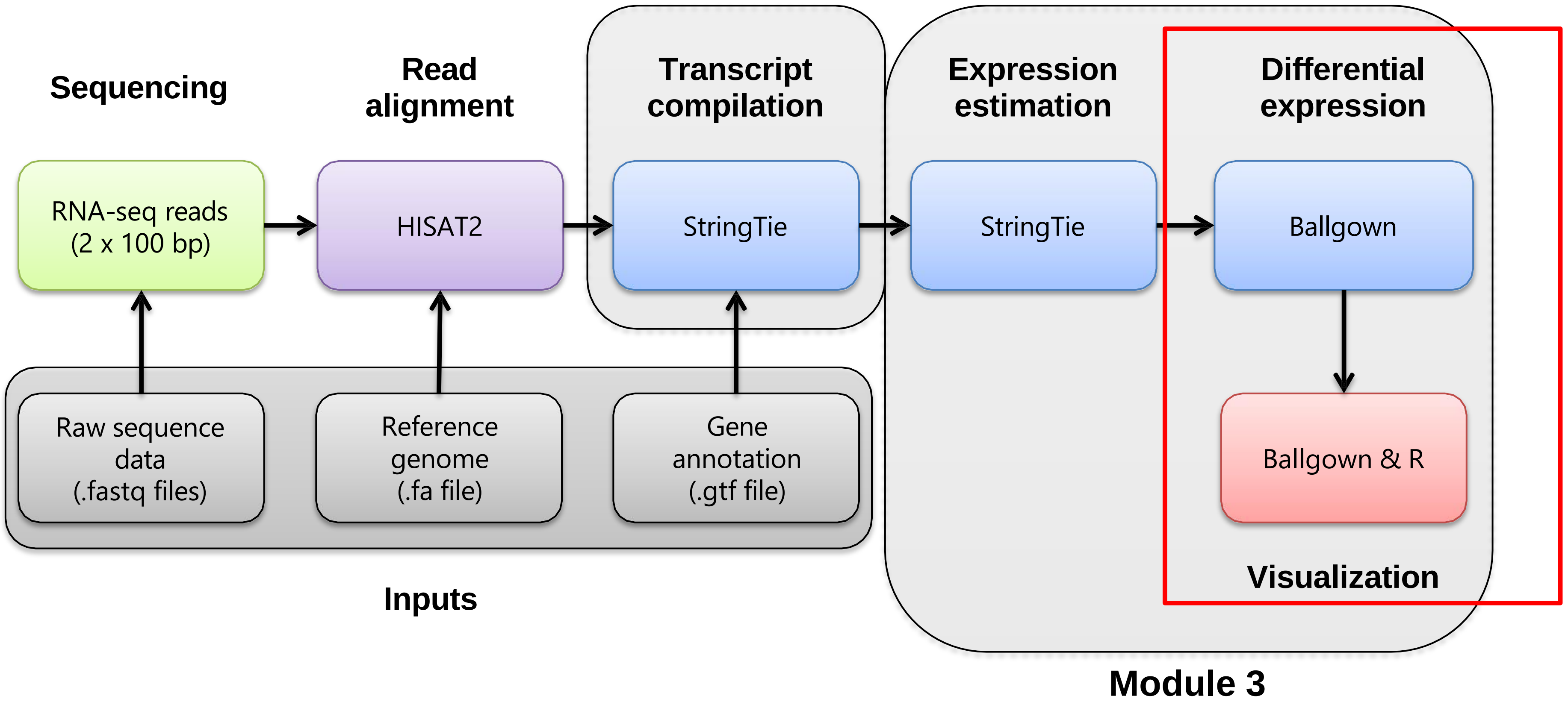
- Make sure to:

  1. Switch User `su` to the `unbutu` user → `su ubuntu`
  2. `source ~/.bashrc`
  3. Set the environment variable →

  ```
  export RNA_HOME=~/workspace/rnaseq
  ```

- Prepare input data (if you are stuck, download from: [http://genomedata.org/rnaseq- tutorial/results/cshl2022/rnaseq.tar.gz](http://genomedata.org/rnaseq-tutorial/results/cshl2022/rnaseq.tar.gz))

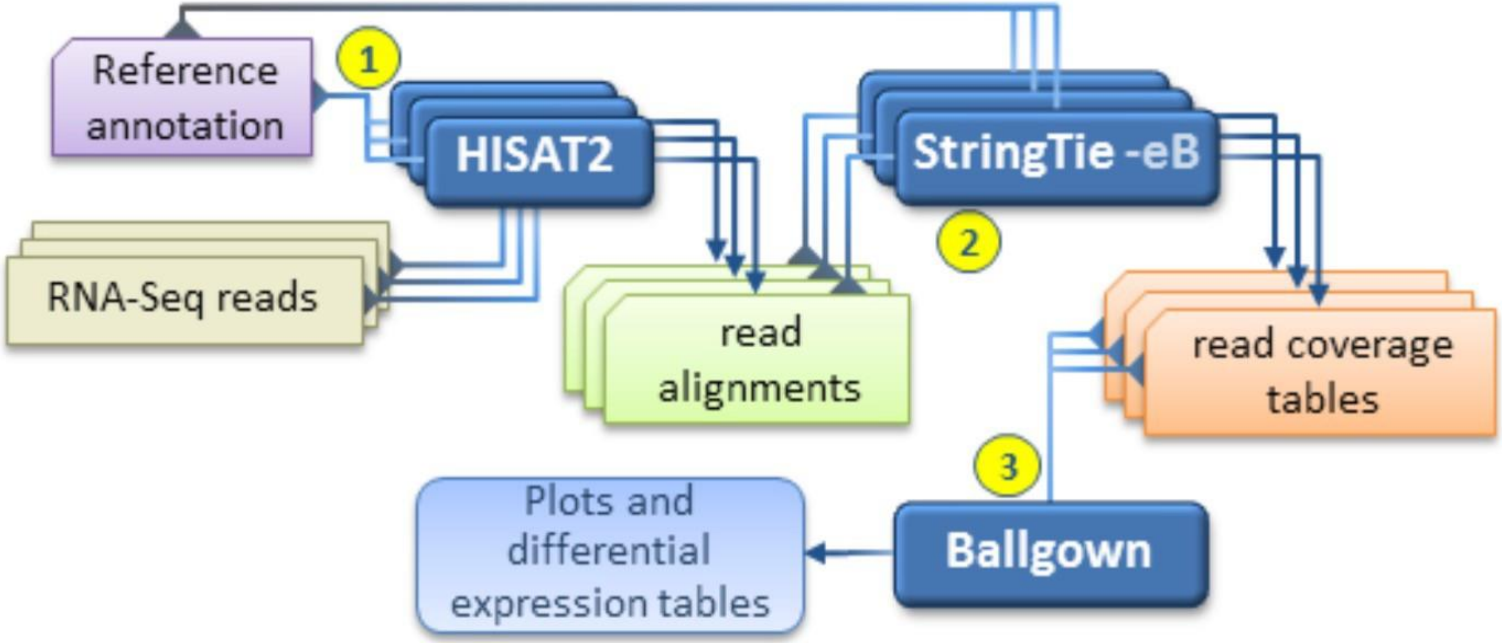
HISAT2/StringTie/Ballgown RNA-seq Pipeline



Last week: Expression estimation (StringTie, htseq count)

This week: Differential expression (Ballgown, edgeR)

Stringtie_Expression Estimate



This is the workflow we used in last week's exercise:
StringTie –G and -e

Expression estimation mode ("Reference Only")

Summary

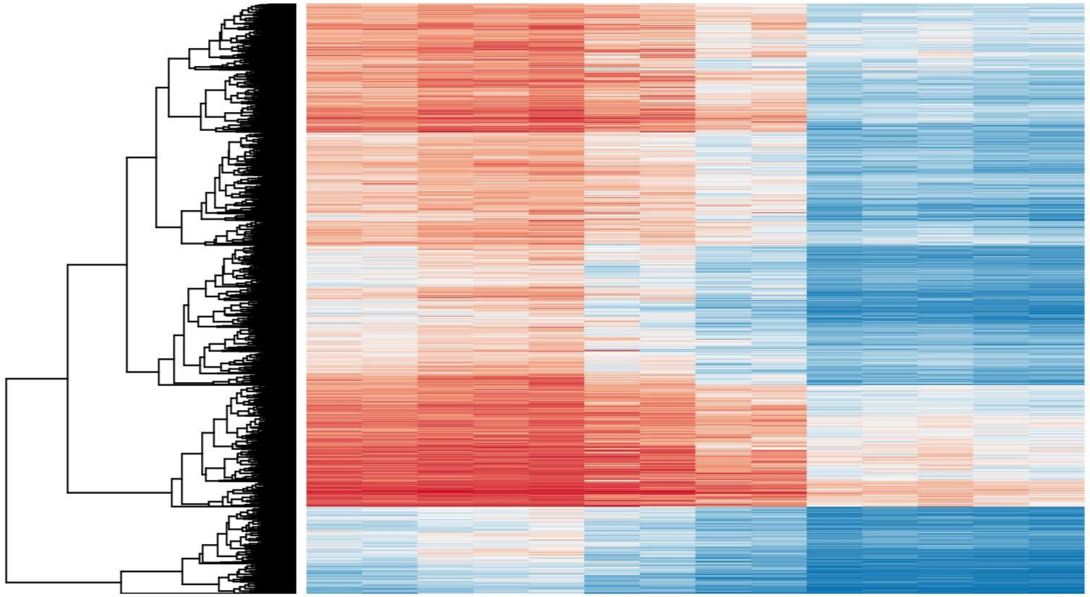
- Normalized counts account for sequencing depth and gene length biases
  - RPKM ~ single-end sequencing, FPKM ~ paired-end sequencing
  - The sum of all TPMs in each sample is the same. Easier to compare across samples!
- Abundance estimation tool that calculates normalized count (FPKM, TPM): StringTie
- Abundance estimation tool that calculates raw count: HTseq

Differential Expression

- Tying gene expression back to genotype/phenotype

- What genes/transcripts are being expressed at higher/lower levels in different groups of samples?
  - Are these differences ‘significant’, accounting for variance/noise?

- Examples (used in course):
  - UHR cells vs HBR brain
  - Tumor vs Normal tissue
  - Wild-type vs gene KO cells



Differential Expression with Ballgown

Parametric F-test comparing nested linear models

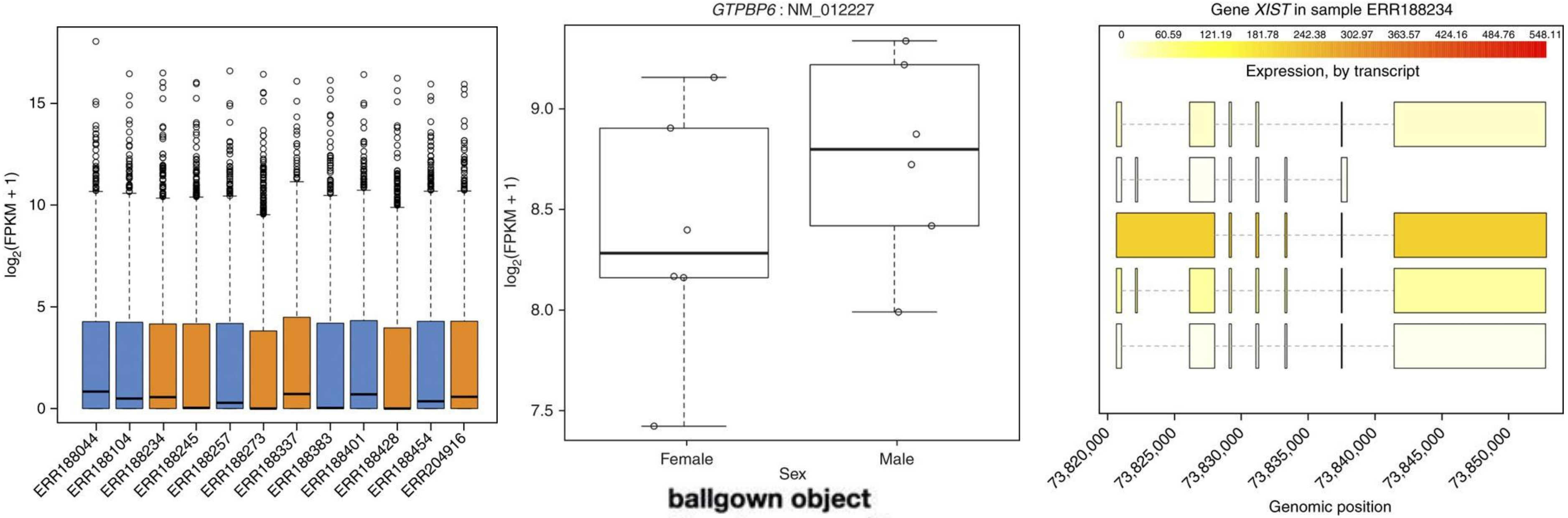
- Two models are fit to each feature, using expression as the outcome
  - one including the covariate of interest (e.g., case/control status or time) and one not including that covariate.

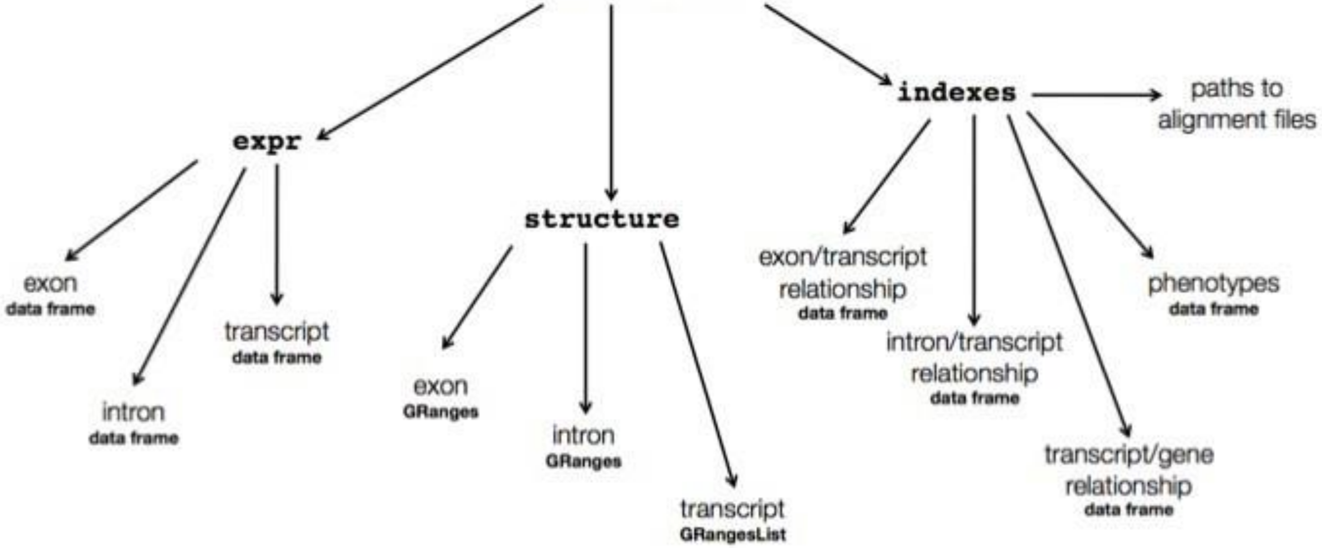
- An F statistic and p-value are calculated using the fits of the two models.
  - A significant p-value means the model including the covariate of interest fits significantly better than the model without that covariate, indicating differential expression.

- Adjust for multiple testing by reporting q-values:
  - $q < 0.05$ the false discovery rate should be controlled at ~5%.

Frazee et al. (2014)

Ballgown for Visualization with R





ballgown object

expr

exon data frame

intron data frame

transcript data frame

structure

exon GRanges

intron GRanges

transcript GRangesList

indexes

paths to alignment files

exon/transcript relationship data frame

intron/transcript relationship data frame

transcript/gene relationship data frame

phenotypes data frame

Alternative differential expression methods

- Raw count approaches
  - DESeq2 - http://www-huber.embl.de/users/anders/DESeq/
  - edgeR - http://www.bioconductor.org/packages/release/bioc/html/edgeR.html
  - Others…

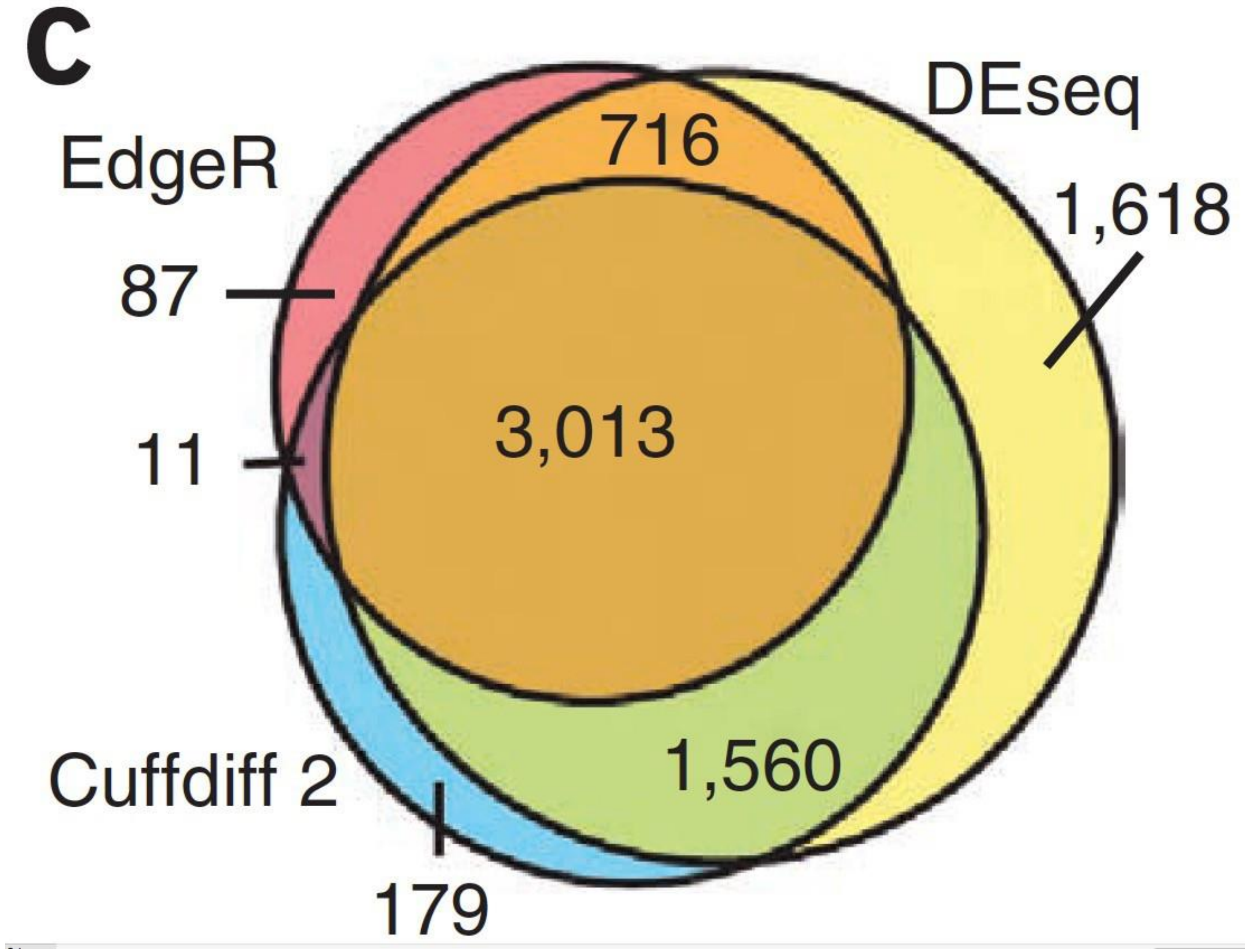
‘FPKM/TPM’ expression estimates vs. ‘raw’ counts

- Which should I use?
  - Long running debate, but the general consensus:

- FPKM/TPM
  - When you want to leverage benefits of tuxedo suite
    - Isoform deconvolution
  - Good for visualization (e.g., heatmaps)
  - Calculating fold changes, etc.

- Counts
  - “More robust" statistical methods for differential expression
    - Stringtie/Ballgown approach is also robust
  - Accommodates more sophisticated experimental designs with appropriate statistical tests

Multiple approaches advisable



Lessons learned from microarray days

- Hansen et al. “Sequencing Technology Does Not Eliminate Biological Variability.” Nature Biotechnology 29, no. 7 (2011): 572–573.

- Power analysis for RNA-seq experiments
  - http://scotty.genetics.utah.edu/

- RNA-seq need for biological replicates
  - http://www.biostars.org/p/1161/

- RNA-seq study design
  - http://www.biostars.org/p/68885/

Multiple testing correction

- As more attributes are compared, differences due solely to chance become more likely!

- Well known from array studies
  - 10,000s genes/transcripts
  - 100,000s exons

- With RNA-seq, more of a problem than ever
  - All the complexity of the transcriptome gives huge numbers of potential features
    - Genes, transcripts, exons, junctions, retained introns, microRNAs, lncRNAs, etc

- Bioconductor multtest
  - http://www.bioconductor.org/packages/release/bioc/html/multtest.html

Downstream interpretation of expression analysis

- Topic for an entire course
- Expression estimates and differential expression lists from StringTie, Ballgown or other alternatives can be fed into many analysis pipelines
- See supplemental R tutorial for how to format expression data and start manipulating in R

- Clustering/Heatmaps
  - Provided by Ballgown
  - For more customized analysis various R packages exist:
    - hclust, heatmap.2, plotrix, ggplot2, etc.
- Classification
  - For RNA-seq data we still rarely have sufficient sample size and clinical details but this is changing
    - Weka is a good learning tool
    - RandomForests R package (biostar tutorial being developed)
- Pathway analysis
  - GSEA, IPA, Cytoscape, many R/BioConductor packages: http://www.bioconductor.org/help/search/index.html?q=pathway

https://genviz.org/module-04-expression/0004/01/01/Expression_Profiling_and_Visualization/

RNA Input Data

- Universal Human Reference (UHR) and Human Brain Reference (HBR)
- In addition, a spike-in control was used (ERCC ExFold RNA Spike-In Control Mixes) to each sample.

o UHR + ERCC Spike-In Mix1, Replicate 1

o UHR + ERCC Spike-In Mix1, Replicate 2

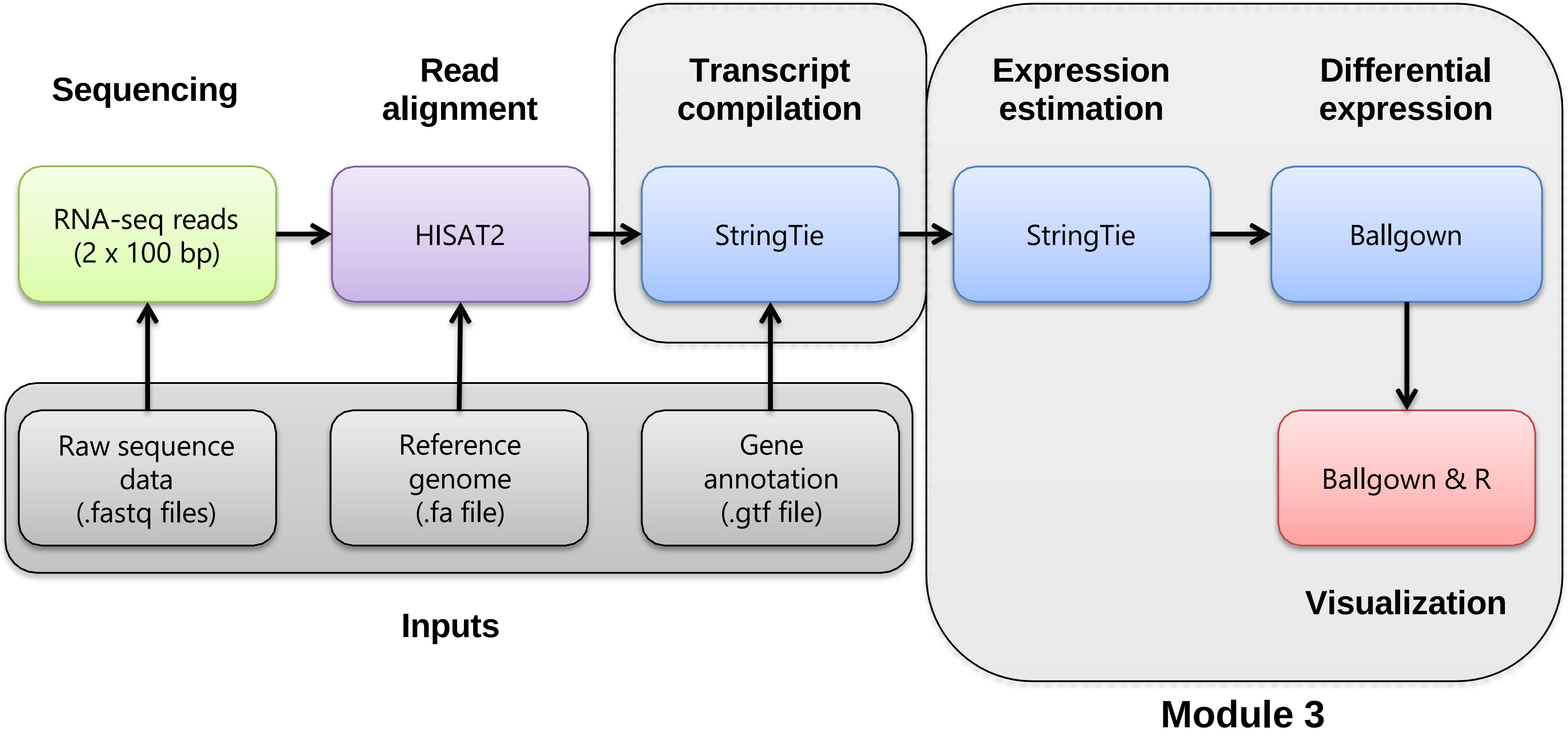
o UHR + ERCC Spike-In Mix1, Replicate 3

o HBR + ERCC Spike-In Mix2, Replicate 1

o HBR + ERCC Spike-In Mix2, Replicate 2

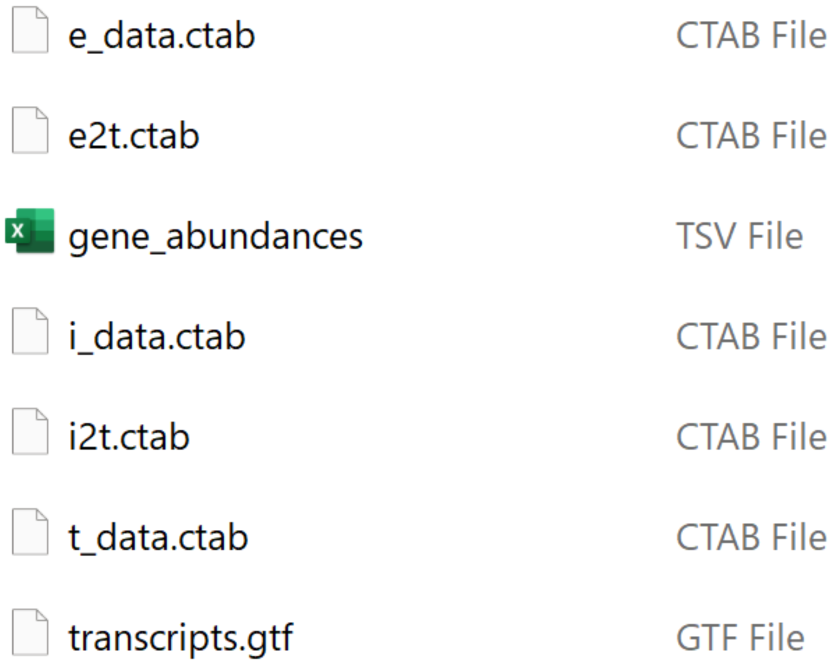
o HBR + ERCC Spike-In Mix2, Replicate 3

HISAT2/StringTie/Ballgown RNA-seq Pipeline



Stringtie outputs

- Stringtie gives 3 metrics for expression levels: coverage, FPKM, TPM ; for 2 types : transcript and gene.
- Focus on the ‘transcript.gtf’ and ‘gene_abundance.tsv’

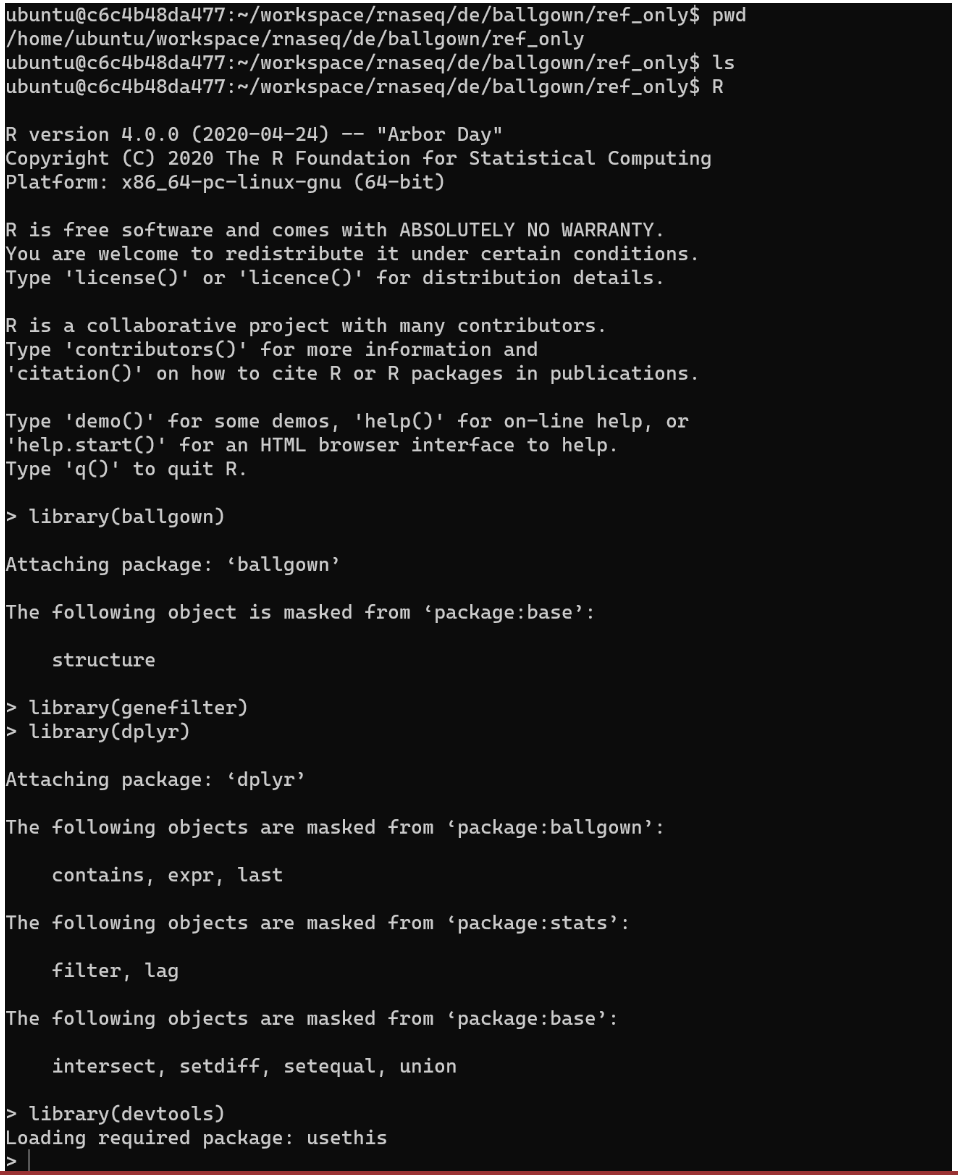


e_data.ctab	CTAB File
e2t.ctab	CTAB File
gene_abundances	TSV File
i_data.ctab	CTAB File
i2t.ctab	CTAB File
t_data.ctab	CTAB File
transcripts.gtf	GTF File

e_data.ctab: Contains information about exon-level expression
i_data.ctab: Contains information about intron-level expression (used less frequently in expression analysis)
t_data.ctab: Contains information about transcript-level expression
e2t.ctab, i2t.ctab: These ‘edge’ files map relationships between transcripts and exons/introns
gene_abundances.tsv: A tab-separated file containing gene-level expression estimates, typically including FPKM values
transcripts.gtf: The assembled transcripts file in GTF format that includes the estimated transcript structures and their expression levels

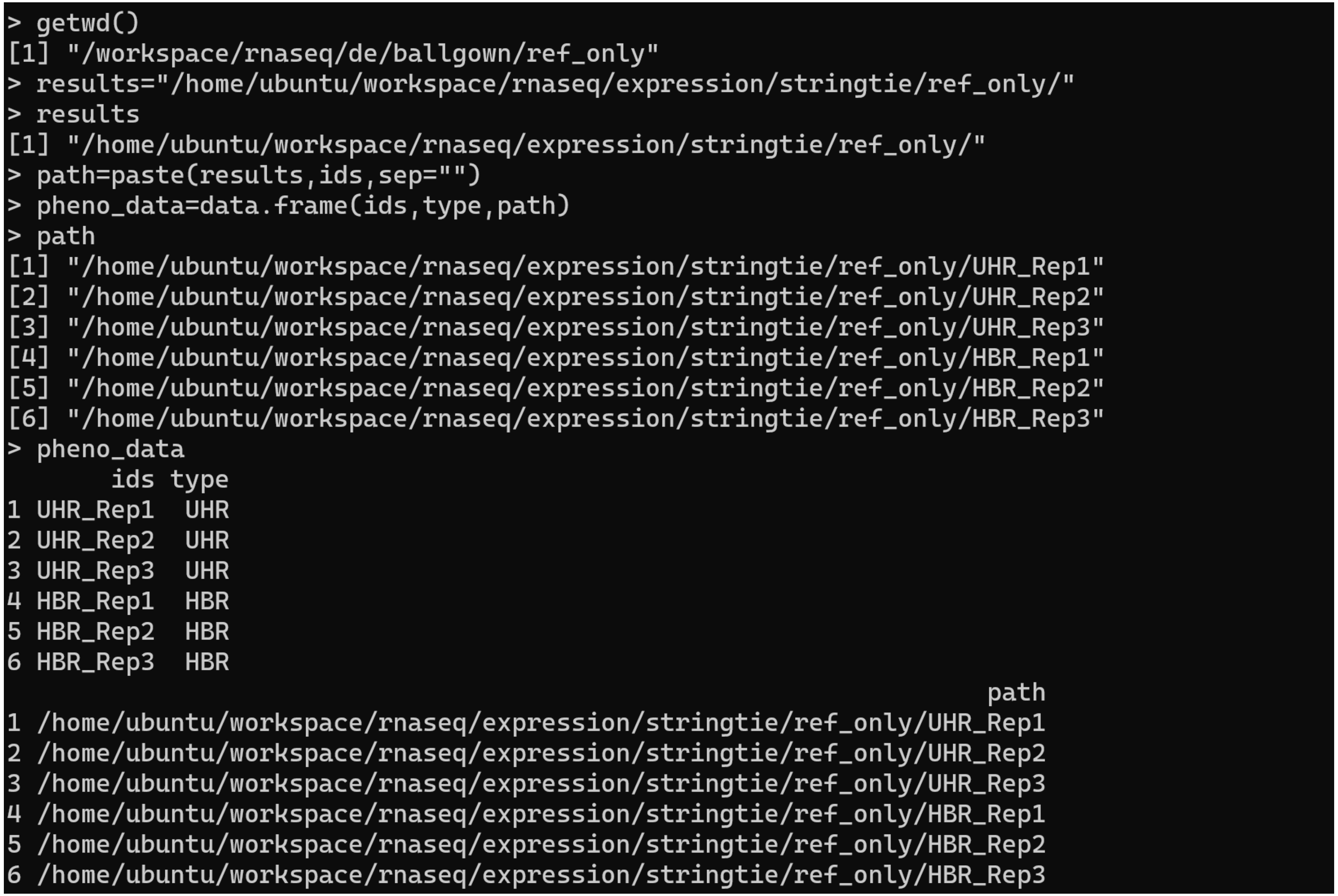
Assignment: Ballgown DE Analysis

- Run R and load required libraries



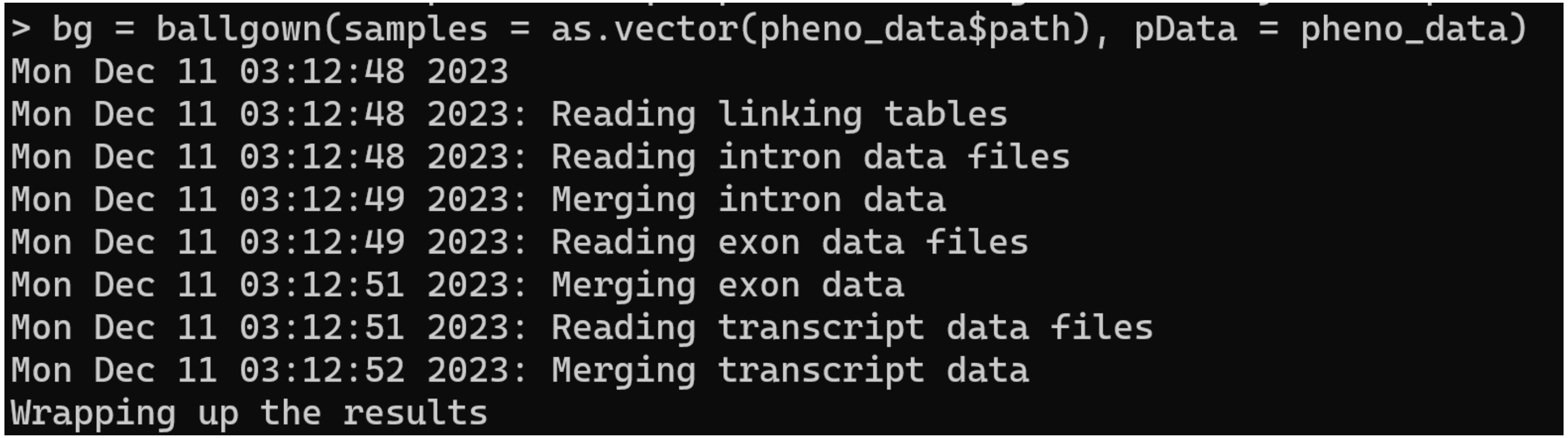
```
ubuntu@c6c4b48da477:~/workspace/rnaseq/de/ballgown/ref_only$ pwd
/home/ubuntu/workspace/rnaseq/de/ballgown/ref_only
ubuntu@c6c4b48da477:~/workspace/rnaseq/de/ballgown/ref_only$ ls
ubuntu@c6c4b48da477:~/workspace/rnaseq/de/ballgown/ref_only$ R

R version 4.0.0 (2020-04-24) -- "Arbor Day"
Copyright (C) 2020 The R Foundation for Statistical Computing
Platform: x86_64-pc-linux-gnu (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

> library(ballgown)

Attaching package: ‘ballgown’

The following object is masked from ‘package:base’:

    structure

> library(genefilter)
> library(dplyr)

Attaching package: ‘dplyr’

The following objects are masked from ‘package:ballgown’:

    contains, expr, last

The following objects are masked from ‘package:stats’:

    filter, lag

The following objects are masked from ‘package:base’:

    intersect, setdiff, setequal, union

> library(devtools)
Loading required package: usethis
>
```

Create phenotype data needed for ballgown analysis

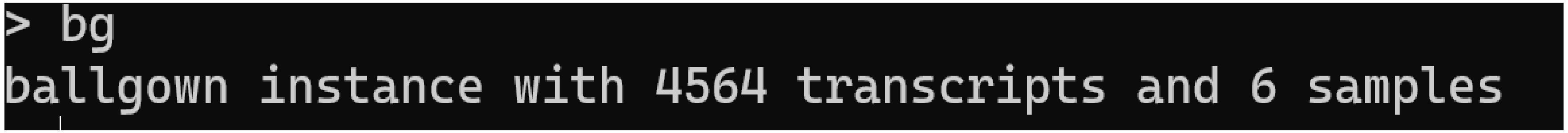


```
> getwd()
[1] "/workspace/rnaseq/de/ballgown/ref_only"
> results="/home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/"
> results
[1] "/home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/"
> path=paste(results,ids,sep="")
> pheno_data=data.frame(ids,type,path)
> path
[1] "/home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/UHR_Rep1"
[2] "/home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/UHR_Rep2"
[3] "/home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/UHR_Rep3"
[4] "/home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/HBR_Rep1"
[5] "/home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/HBR_Rep2"
[6] "/home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/HBR_Rep3"
> pheno_data
       ids type
1 UHR_Rep1  UHR
2 UHR_Rep2  UHR
3 UHR_Rep3  UHR
4 HBR_Rep1  HBR
5 HBR_Rep2  HBR
6 HBR_Rep3  HBR
                                                                  path
1 /home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/UHR_Rep1
2 /home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/UHR_Rep2
3 /home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/UHR_Rep3
4 /home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/HBR_Rep1
5 /home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/HBR_Rep2
6 /home/ubuntu/workspace/rnaseq/expression/stringtie/ref_only/HBR_Rep3
```

Create the ballgown data structure



```
> bg = ballgown(samples = as.vector(pheno_data$path), pData = pheno_data)
Mon Dec 11 03:12:48 2023
Mon Dec 11 03:12:48 2023: Reading linking tables
Mon Dec 11 03:12:48 2023: Reading intron data files
Mon Dec 11 03:12:49 2023: Merging intron data
Mon Dec 11 03:12:49 2023: Reading exon data files
Mon Dec 11 03:12:51 2023: Merging exon data
Mon Dec 11 03:12:51 2023: Reading transcript data files
Mon Dec 11 03:12:52 2023: Merging transcript data
Wrapping up the results
```

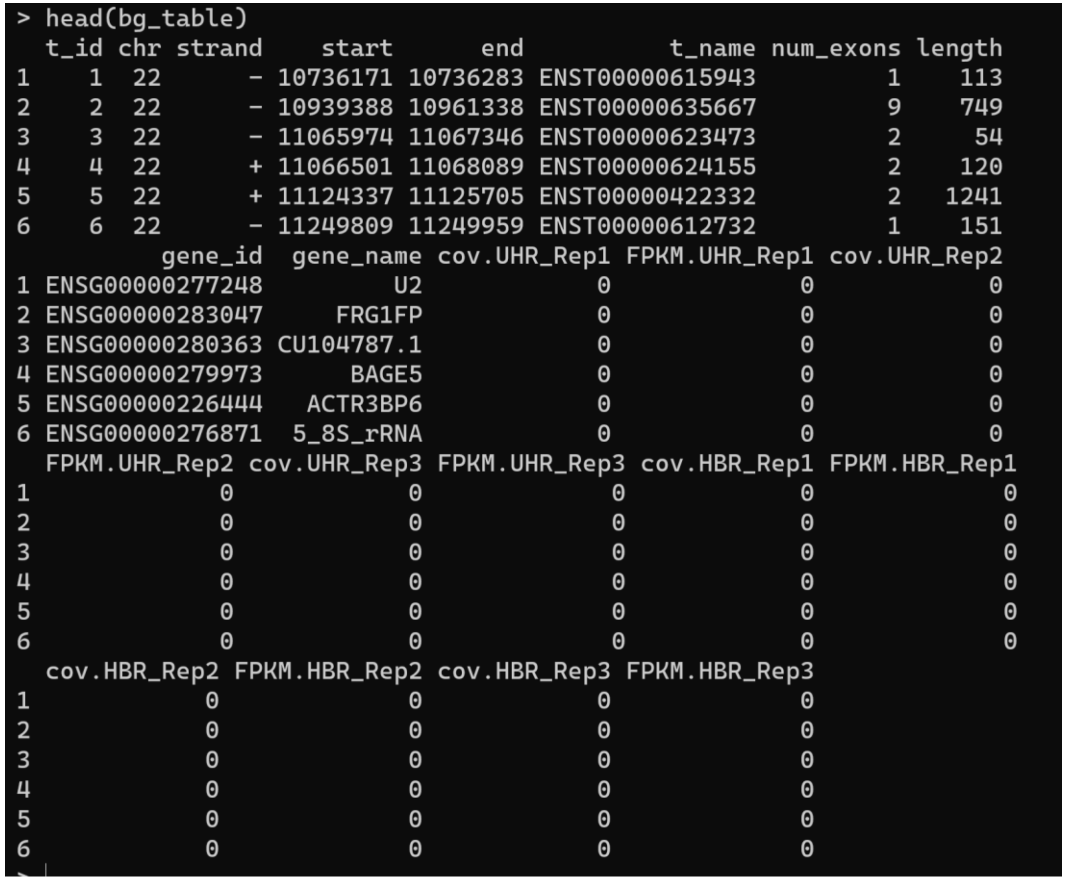


```
> bg
ballgown instance with 4564 transcripts and 6 samples
```

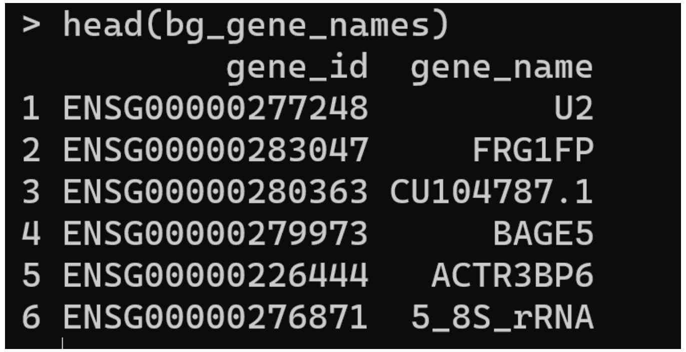
Verify your object

Attributes

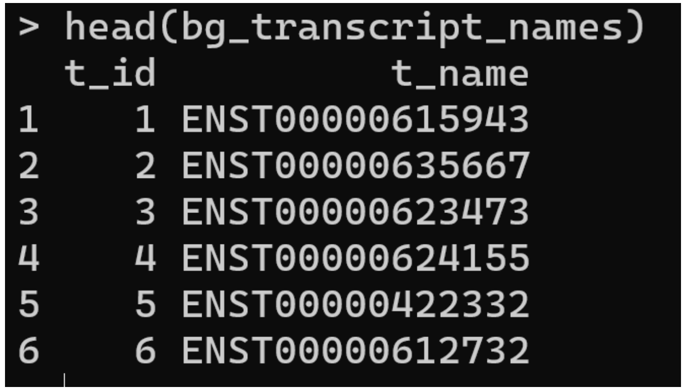
- Extract all transcript-level expression data from the bg object. Then extract unique gene and unique transcript IDs.



```
> head(bg_table)
  t_id chr strand    start      end          t_name num_exons length
1    1  22      - 10736171 10736283 ENST00000615943         1    113
2    2  22      - 10939388 10961338 ENST00000635667         9    749
3    3  22      - 11065974 11067346 ENST00000623473         2     54
4    4  22      + 11066501 11068089 ENST00000624155         2    120
5    5  22      + 11124337 11125705 ENST00000422332         2   1241
6    6  22      - 11249809 11249959 ENST00000612732         1    151
          gene_id  gene_name cov.UHR_Rep1 FPKM.UHR_Rep1 cov.UHR_Rep2
1 ENSG00000277248         U2            0             0            0
2 ENSG00000283047     FRG1FP            0             0            0
3 ENSG00000280363 CU104787.1            0             0            0
4 ENSG00000279973      BAGE5            0             0            0
5 ENSG00000226444   ACTR3BP6            0             0            0
6 ENSG00000276871  5_8S_rRNA            0             0            0
  FPKM.UHR_Rep2 cov.UHR_Rep3 FPKM.UHR_Rep3 cov.HBR_Rep1 FPKM.HBR_Rep1
1             0            0             0            0             0
2             0            0             0            0             0
3             0            0             0            0             0
4             0            0             0            0             0
5             0            0             0            0             0
6             0            0             0            0             0
  cov.HBR_Rep2 FPKM.HBR_Rep2 cov.HBR_Rep3 FPKM.HBR_Rep3
1            0             0            0             0
2            0             0            0             0
3            0             0            0             0
4            0             0            0             0
5            0             0            0             0
6            0             0            0             0
```



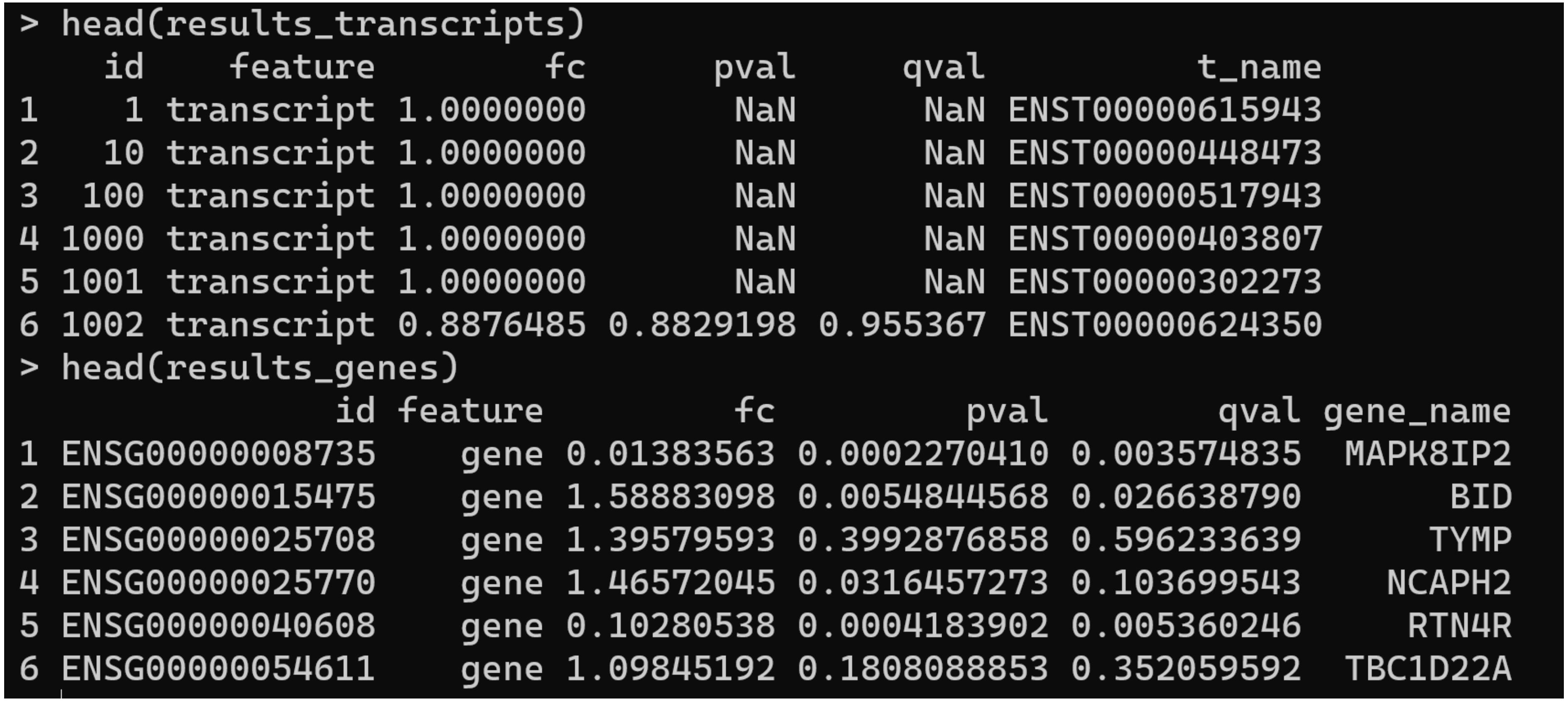
```
> head(bg_gene_names)
          gene_id  gene_name
1 ENSG00000277248         U2
2 ENSG00000283047     FRG1FP
3 ENSG00000280363 CU104787.1
4 ENSG00000279973      BAGE5
5 ENSG00000226444   ACTR3BP6
6 ENSG00000276871  5_8S_rRNA
```



```
> head(bg_transcript_names)
  t_id          t_name
1    1 ENST00000615943
2    2 ENST00000635667
3    3 ENST00000623473
4    4 ENST00000624155
5    5 ENST00000422332
6    6 ENST00000612732
```

Perform DE analysis without filtering

```
results_transcripts = stattest(bg, feature="transcript", covariate="type",
getFC=TRUE, meas="FPKM")

results_transcripts = merge(results_transcripts, bg_transcript_names, by.x=c("id"),
by.y=c("t_id"))
```



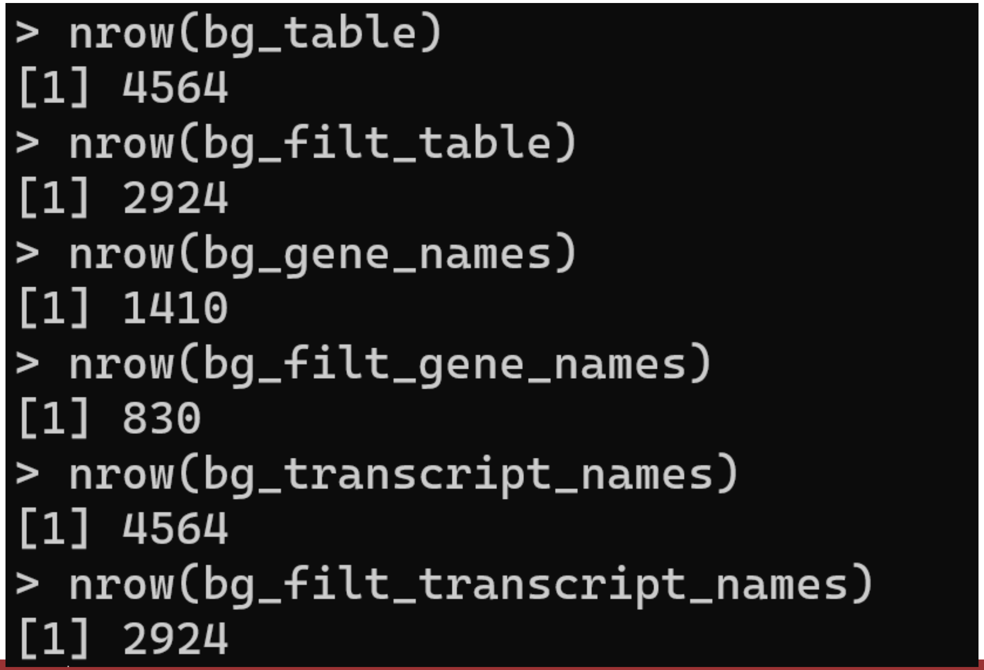
```
> head(results_transcripts)
    id    feature        fc      pval     qval          t_name
1    1 transcript 1.0000000       NaN      NaN ENST00000615943
2   10 transcript 1.0000000       NaN      NaN ENST00000448473
3  100 transcript 1.0000000       NaN      NaN ENST00000517943
4 1000 transcript 1.0000000       NaN      NaN ENST00000403807
5 1001 transcript 1.0000000       NaN      NaN ENST00000302273
6 1002 transcript 0.8876485 0.8829198 0.955367 ENST00000624350
> head(results_genes)
               id feature         fc         pval        qval gene_name
1 ENSG00000008735    gene 0.01383563 0.0002270410 0.003574835  MAPK8IP2
2 ENSG00000015475    gene 1.58883098 0.0054844568 0.026638790       BID
3 ENSG00000025708    gene 1.39579593 0.3992876858 0.596233639      TYMP
4 ENSG00000025770    gene 1.46572045 0.0316457273 0.103699543    NCAPH2
5 ENSG00000040608    gene 0.10280538 0.0004183902 0.005360246     RTN4R
6 ENSG00000054611    gene 1.09845192 0.1808088853 0.352059592  TBC1D22A
```

Filter low-abundance genes

`subset():` This function subsets the bg object to include only those transcripts with a variance across the samples greater than 1.
This step is designed to remove transcripts that do not show much change across your conditions, under the assumption that they are not likely to be biologically interesting

```
bg_filt = subset (bg,"rowVars(texpr(bg)) > 1", genomesubset=TRUE)
```

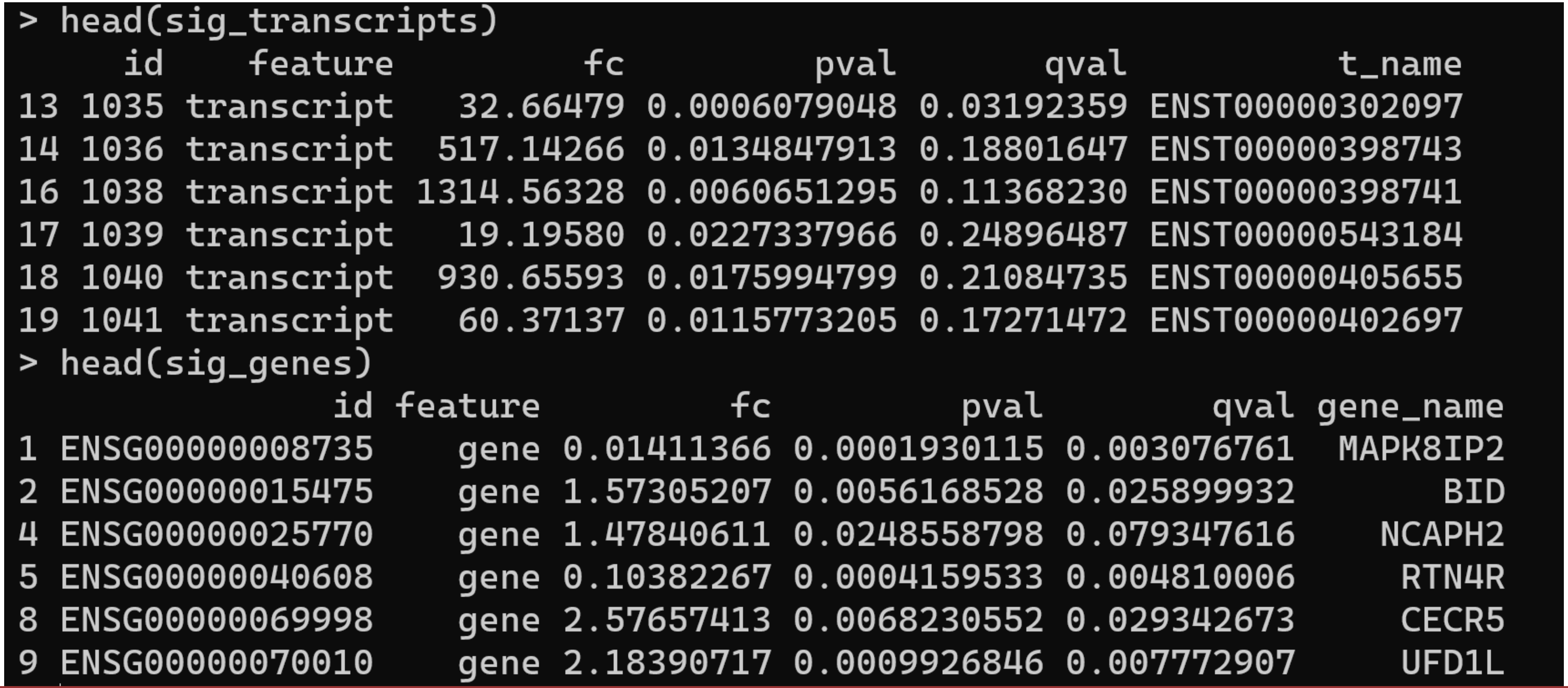
where genomesubset=TRUE → ensures that when you subset the transcripts, the associated genomic features (like exons and introns) are also appropriately subsetted.



```
> nrow(bg_table)
[1] 4564
> nrow(bg_filt_table)
[1] 2924
> nrow(bg_gene_names)
[1] 1410
> nrow(bg_filt_gene_names)
[1] 830
> nrow(bg_transcript_names)
[1] 4564
> nrow(bg_filt_transcript_names)
[1] 2924
```

Perform DE analysis using the filtered data and identify significant genes

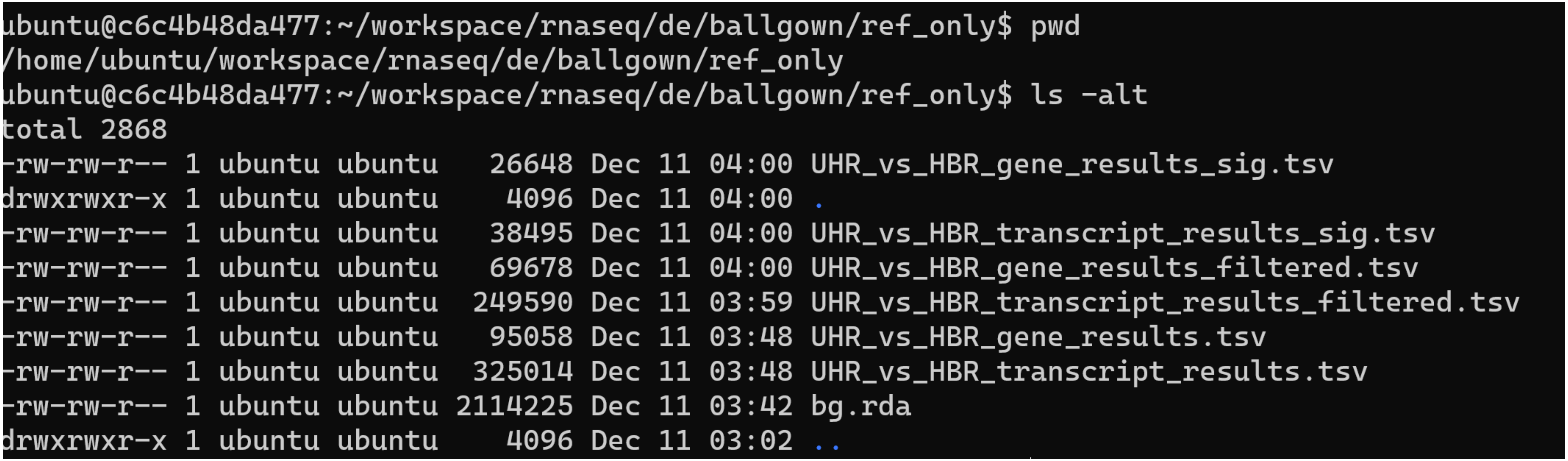
```
sig_transcripts = subset(results_transcripts, results_transcripts$pval<0.05)
sig_genes = subset(results_genes, results_genes$pval<0.05)
```



```
> head(sig_transcripts)
     id    feature         fc         pval       qval          t_name
13 1035 transcript   32.66479 0.0006079048 0.03192359 ENST00000302097
14 1036 transcript  517.14266 0.0134847913 0.18801647 ENST00000398743
16 1038 transcript 1314.56328 0.0060651295 0.11368230 ENST00000398741
17 1039 transcript   19.19580 0.0227337966 0.24896487 ENST00000543184
18 1040 transcript  930.65593 0.0175994799 0.21084735 ENST00000405655
19 1041 transcript   60.37137 0.0115773205 0.17271472 ENST00000402697
> head(sig_genes)
               id feature         fc         pval        qval gene_name
1 ENSG00000008735    gene 0.01411366 0.0001930115 0.003076761  MAPK8IP2
2 ENSG00000015475    gene 1.57305207 0.0056168528 0.025899932       BID
4 ENSG00000025770    gene 1.47840611 0.0248558798 0.079347616    NCAPH2
5 ENSG00000040608    gene 0.10382267 0.0004159533 0.004810006     RTN4R
8 ENSG00000069998    gene 2.57657413 0.0068230552 0.029342673     CECR5
9 ENSG00000070010    gene 2.18390717 0.0009926846 0.007772907     UFD1L
```

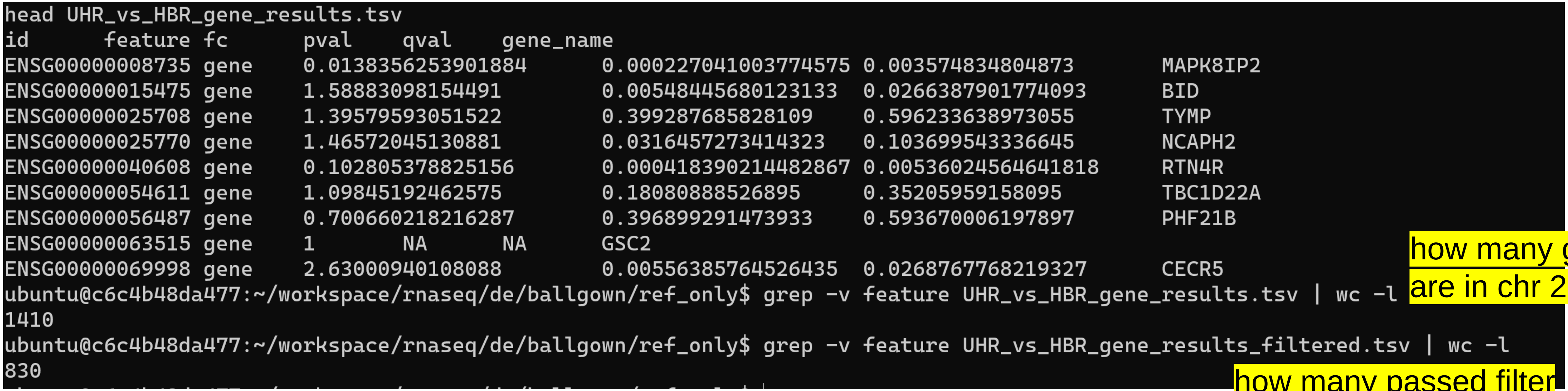
Expected output in

$RNA_HOME/de/ballgown/ref_only/



```
ubuntu@c6c4b48da477:~/workspace/rnaseq/de/ballgown/ref_only$ pwd
/home/ubuntu/workspace/rnaseq/de/ballgown/ref_only
ubuntu@c6c4b48da477:~/workspace/rnaseq/de/ballgown/ref_only$ ls -alt
total 2868
-rw-rw-r-- 1 ubuntu ubuntu   26648 Dec 11 04:00 UHR_vs_HBR_gene_results_sig.tsv
drwxrwxr-x 1 ubuntu ubuntu    4096 Dec 11 04:00 .
-rw-rw-r-- 1 ubuntu ubuntu   38495 Dec 11 04:00 UHR_vs_HBR_transcript_results_sig.tsv
-rw-rw-r-- 1 ubuntu ubuntu   69678 Dec 11 04:00 UHR_vs_HBR_gene_results_filtered.tsv
-rw-rw-r-- 1 ubuntu ubuntu  249590 Dec 11 03:59 UHR_vs_HBR_transcript_results_filtered.tsv
-rw-rw-r-- 1 ubuntu ubuntu   95058 Dec 11 03:48 UHR_vs_HBR_gene_results.tsv
-rw-rw-r-- 1 ubuntu ubuntu  325014 Dec 11 03:48 UHR_vs_HBR_transcript_results.tsv
-rw-rw-r-- 1 ubuntu ubuntu 2114225 Dec 11 03:42 bg.rda
drwxrwxr-x 1 ubuntu ubuntu    4096 Dec 11 03:02 ..
```

Exploring ballgown otutputs



```
head UHR_vs_HBR_gene_results.tsv
id      feature fc      pval    qval    gene_name
ENSG00000008735 gene    0.0138356253901884      0.000227041003774575 0.003574834804873       MAPK8IP2
ENSG00000015475 gene    1.58883098154491        0.00548445680123133  0.0266387901774093      BID
ENSG00000025708 gene    1.39579593051522        0.399287685828109    0.596233638973055       TYMP
ENSG00000025770 gene    1.46572045130881        0.0316457273414323   0.103699543336645       NCAPH2
ENSG00000040608 gene    0.102805378825156       0.000418390214482867 0.00536024564641818     RTN4R
ENSG00000054611 gene    1.09845192462575        0.18080888526895     0.35205959158095        TBC1D22A
ENSG00000056487 gene    0.700660218216287       0.396899291473933    0.593670006197897       PHF21B
ENSG00000063515 gene    1       NA      NA      GSC2
ENSG00000069998 gene    2.63000940108088        0.00556385764526435  0.0268767768219327      CECR5
ubuntu@c6c4b48da477:~/workspace/rnaseq/de/ballgown/ref_only$ grep -v feature UHR_vs_HBR_gene_results.tsv | wc -l
1410
ubuntu@c6c4b48da477:~/workspace/rnaseq/de/ballgown/ref_only$ grep -v feature UHR_vs_HBR_gene_results_filtered.tsv | wc -l
830
```

how many genes are in chr 22?

how many passed filter

Purpose of ERCC in RNAs and RNA-Seq Analysis

.Normalization: control for variations in RNA input, reverse transcription efficiency, PCR amplification biases, and sequencing depth

.Validation: provide a way to assess the accuracy of RNA-Seq measurements by comparing the observec fold changes to the known, expected fold changes

.Quality Control: used to check the overall performance of the RNA-Seq workflow, from library preparation to sequencing and data analysis

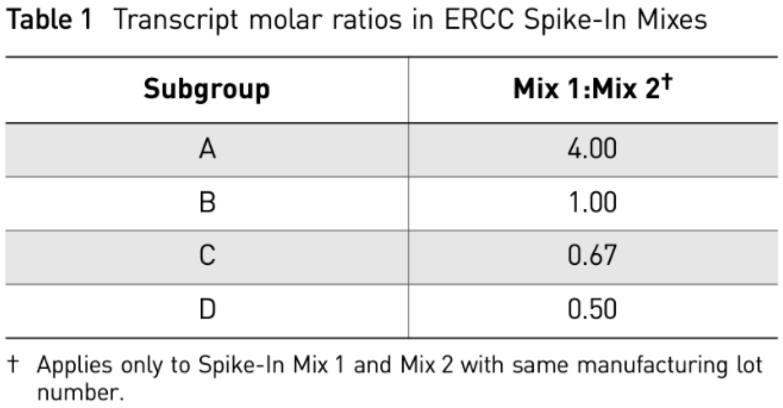
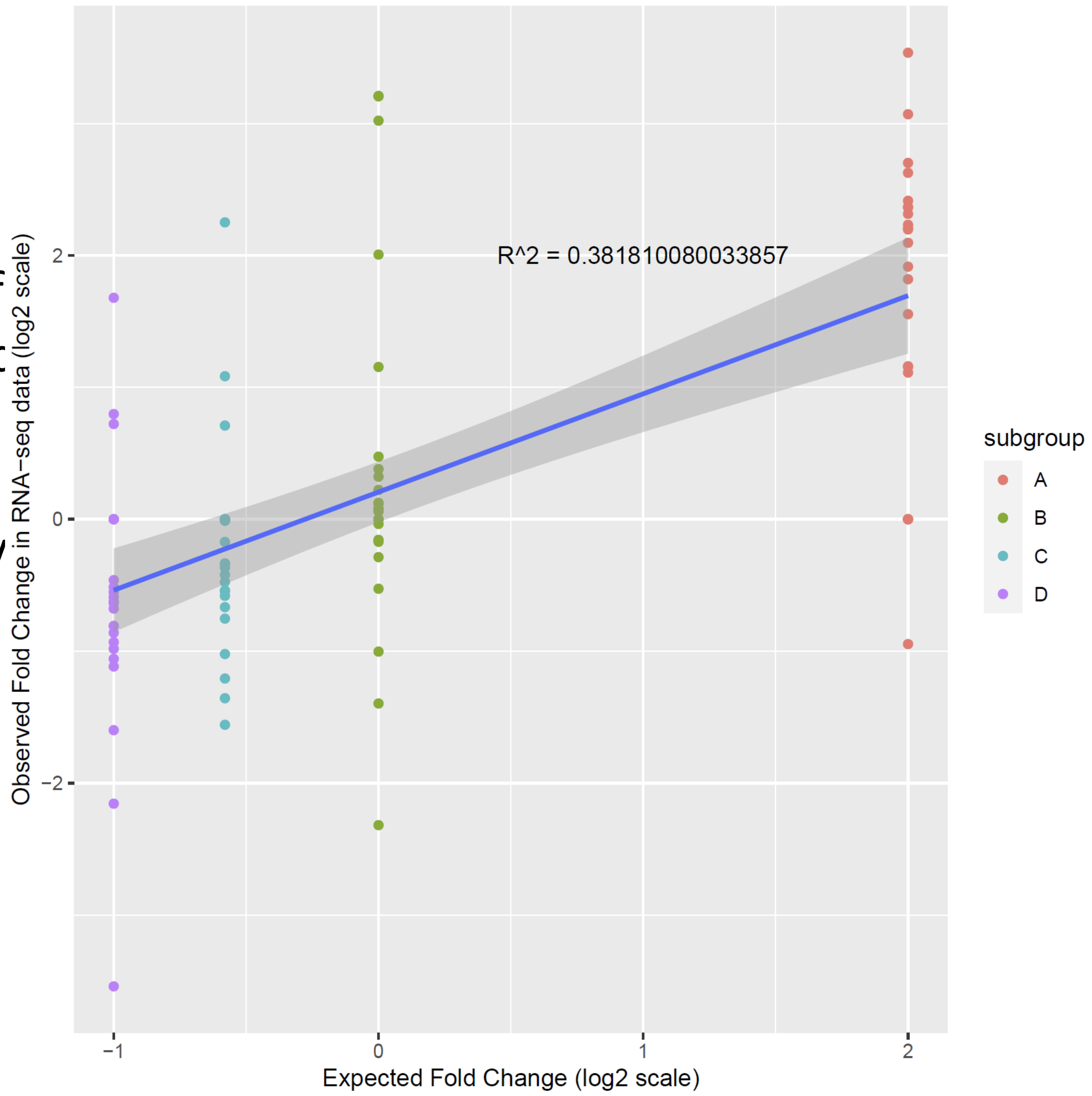


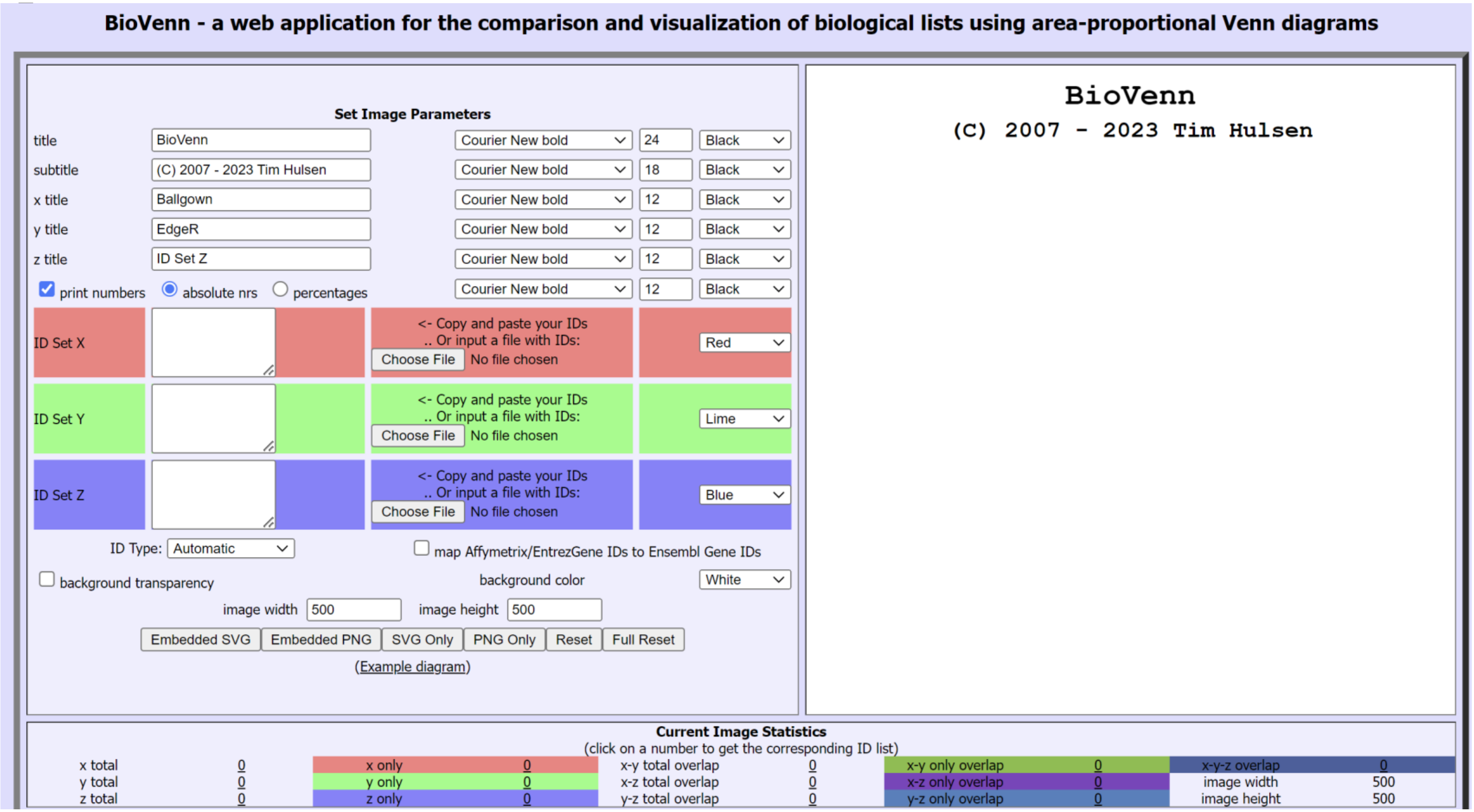
Table 1 Transcript molar ratios in ERCC Spike-In Mixes

Subgroup	Mix 1:Mix 2†
A	4.00
B	1.00
C	0.67
D	0.50

† Applies only to Spike-In Mix 1 and Mix 2 with same manufacturing lot number.



DE Method Comparison (Venn Diagram (DE genes from StringTie/Ballgown vs HTSeq/EdgeR)



BioVenn - a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams

Set Image Parameters

title	BioVenn	Courier New bold	24	Black
subtitle	(C) 2007 - 2023 Tim Hulsen	Courier New bold	18	Black
x title	Ballgown	Courier New bold	12	Black
y title	EdgeR	Courier New bold	12	Black
z title	ID Set Z	Courier New bold	12	Black
☑ print numbers	● absolute nrs ○ percentages	Courier New bold	12	Black
ID Set X		<- Copy and paste your IDs .. Or input a file with IDs: Choose File No file chosen		Red
ID Set Y		<- Copy and paste your IDs .. Or input a file with IDs: Choose File No file chosen		Lime
ID Set Z		<- Copy and paste your IDs .. Or input a file with IDs: Choose File No file chosen		Blue

ID Type: Automatic □ map Affymetrix/EntrezGene IDs to Ensembl Gene IDs

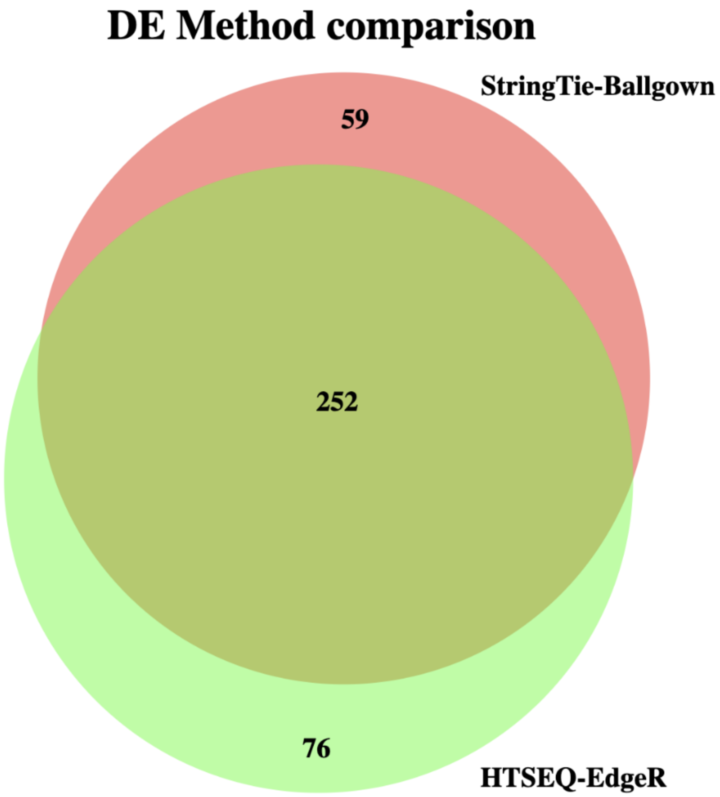
□ background transparency background color White

image width 500 image height 500

BioVenn
(C) 2007 - 2023 Tim Hulsen

Current Image Statistics
(click on a number to get the corresponding ID list)

x total	0	x only	0	x-y total overlap	0	x-y only overlap	0	x-y-z overlap	0
y total	0	y only	0	x-z total overlap	0	x-z only overlap	0	image width	500
z total	0	z only	0	y-z total overlap	0	y-z only overlap	0	image height	500



DE Visualization

https://rnabio.org/module-03-expression/0003/04/01/DE_Visualization/

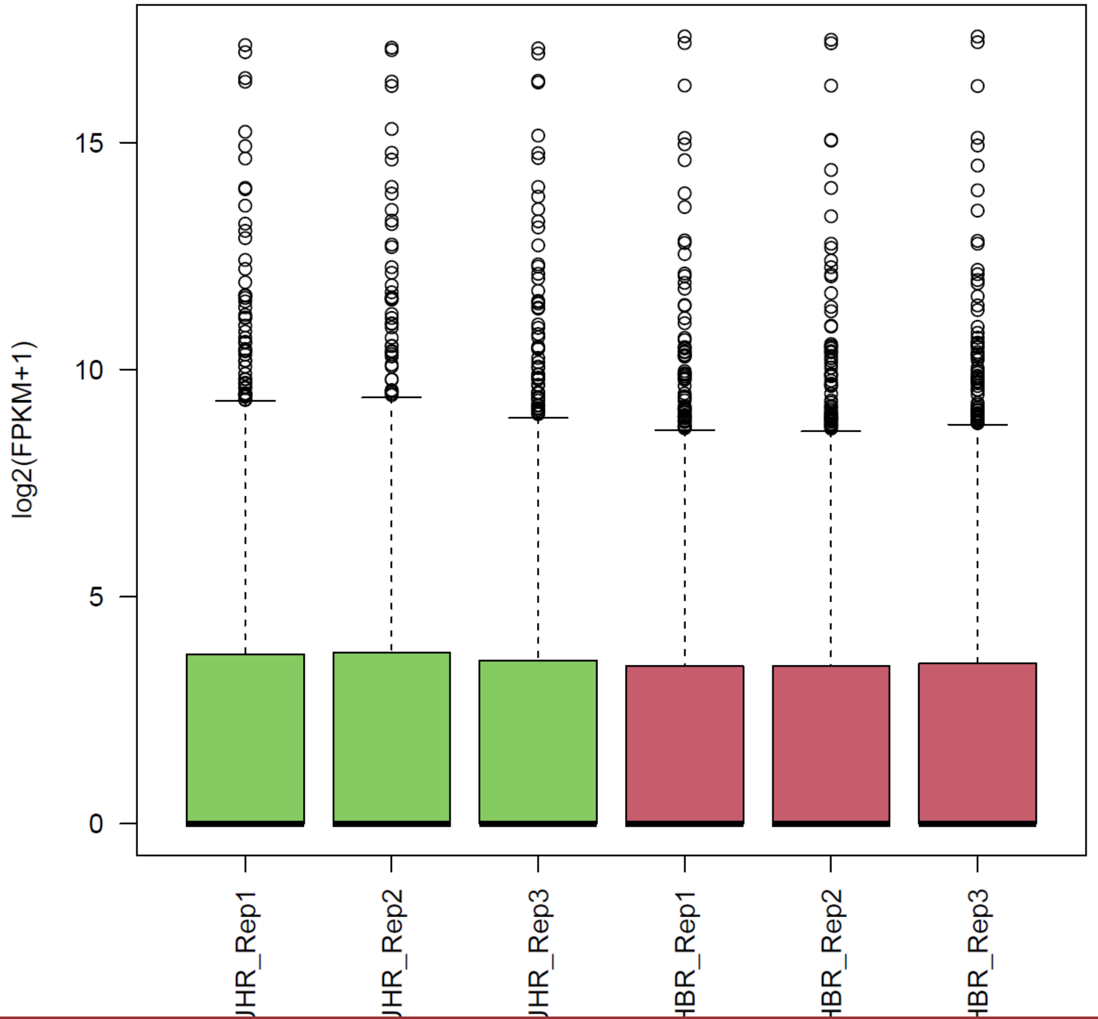
DE_Visualization

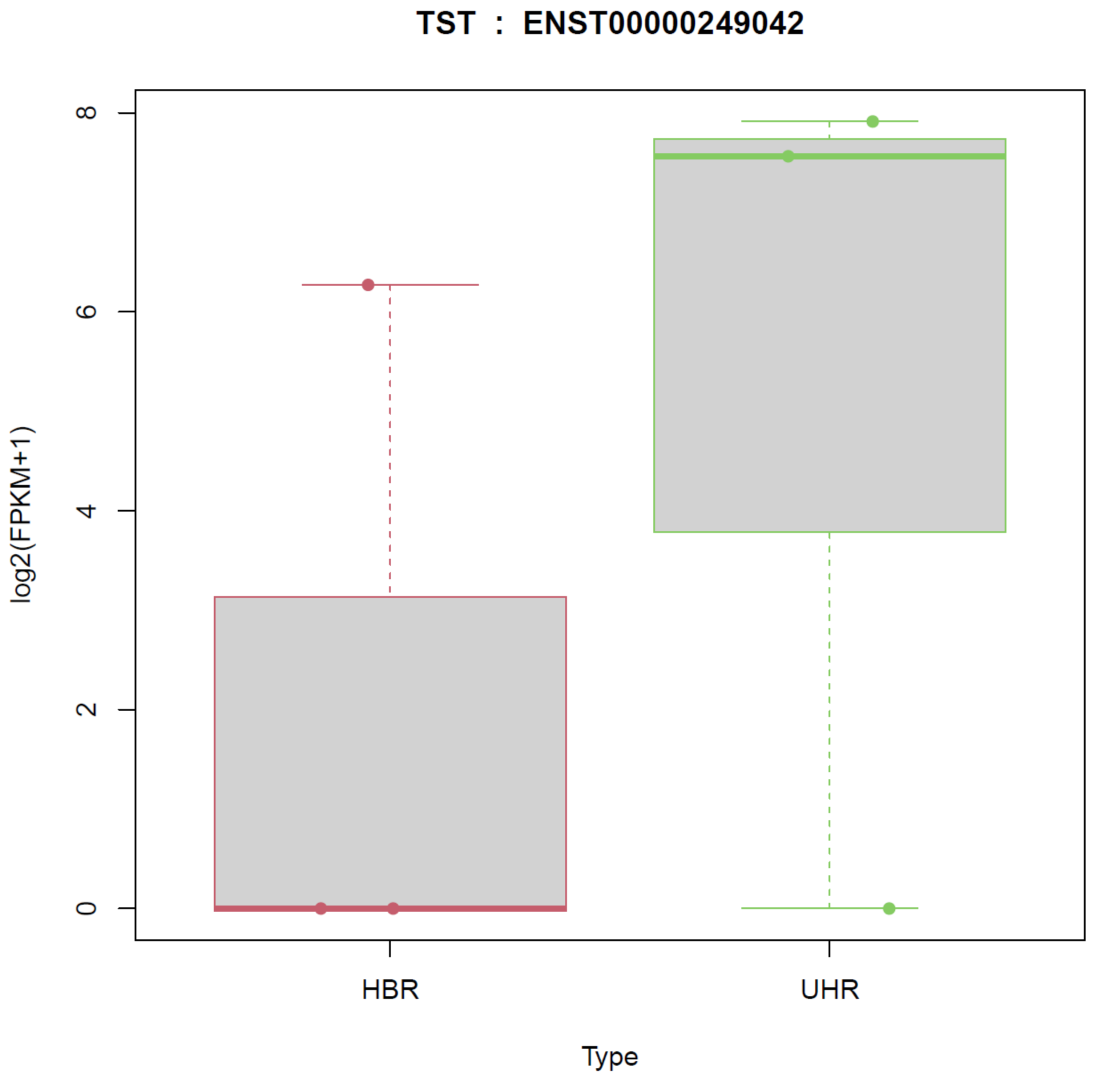
- Phenotype data is loaded along with the Ballgown object (bg.rda), which contains the results of your differential expression analysis

- FPKM values are extracted, log-transformed, and prepared for plotting

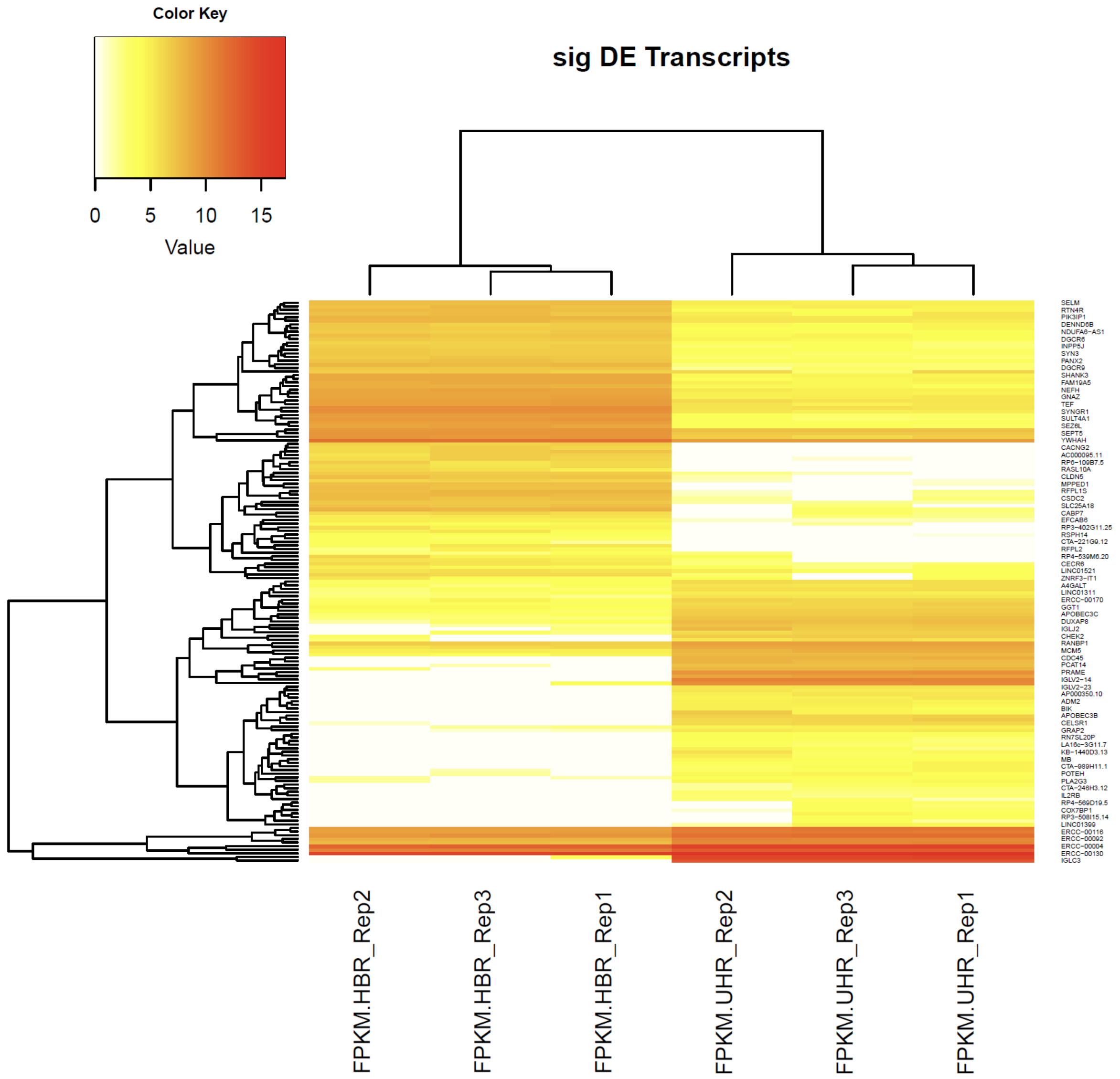
```
fpkm = log2(fpkm+1)
```

FPKM values for each sample/across different types





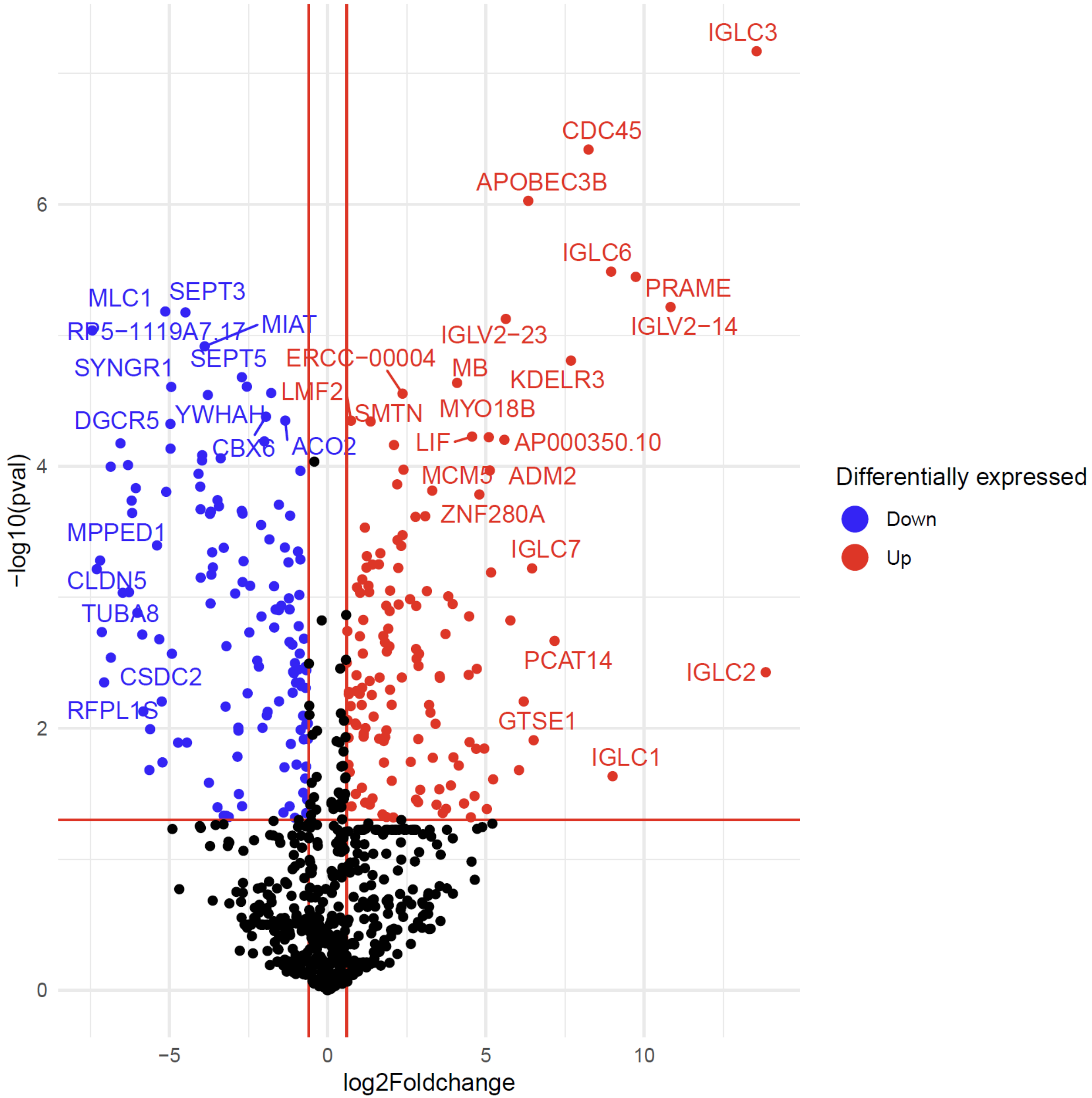
DE_ Heatmap



Color Key
0
5
10
15
Value
sig DE Transcripts
SELM
RTN4R
PIK3IP1
DENND6B
NDUFA6−AS1
DGCR6
INPP5J
SYN3
PANX2
DGCR9
SHANK3
FAM19A5
NEFH
GNAZ
TEF
SYNGR1
SULT4A1
SEZ6L
SEPT5
YWHAH
CACNG2
AC000095.11
RP6−109B7.5
RASL10A
CLDN5
MPPED1
RFPL1S
CSDC2
SLC25A18
CABP7
EFCAB6
RP3−402G11.25
RSPH14
CTA−221G9.12
RFPL2
RP4−539M6.20
CECR6
LINC01521
ZNRF3−IT1
A4GALT
LINC01311
ERCC−00170
GGT1
APOBEC3C
DUXAP8
IGLJ2
CHEK2
RANBP1
MCM5
CDC45
PCAT14
PRAME
IGLV2−14
IGLV2−23
AP000350.10
ADM2
BIK
APOBEC3B
CELSR1
GRAP2
RN7SL20P
LA16c−3G11.7
KB−1440D3.13
MB
CTA−989H11.1
POTEH
PLA2G3
CTA−246H3.12
IL2RB
RP4−569D19.5
COX7BP1
RP3−508I15.14
LINC01399
ERCC−00116
ERCC−00092
ERCC−00004
ERCC−00130
IGLC3
FPKM.HBR_Rep2
FPKM.HBR_Rep3
FPKM.HBR_Rep1
FPKM.UHR_Rep2
FPKM.UHR_Rep3
FPKM.UHR_Rep1

DE_Volcano Plot

UHR vs HBR


IGLC3
CDC45
APOBEC3B
IGLC6
PRAME
IGLV2−14
IGLV2−23
MLC1
SEPT3
RP5−1119A7.17
MIAT
SYNGR1
SEPT5
ERCC−00004
MB
KDELR3
LMF2
DGCR5
YWHAH
SMTN
MYO18B
CBX6
ACO2
LIF
AP000350.10
MCM5
ADM2
ZNF280A
MPPED1
IGLC7
CLDN5
TUBA8
PCAT14
CSDC2
IGLC2
RFPL1S
GTSE1
IGLC1
log2Foldchange
−log10(pval)
Differentially expressed
Down
Up