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Practical section II:
Introduction to R and RNAseq data analysis

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Outline

Part I: Introduction to R

- S1: Data structures and basic operations.
- S2: Data import and export.
- S3: Summary statistics and data visualization.

Part II: Introduction to RNAseq data analysis

- S4: RNAseq data analysis with DESeq2.
- S5: Exploring and visualizing RNAseq data.

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Course materials on GitHub

What is GitHub?

GitHub is like a digital library where people store and share their writing projects, allowing others to view, discuss, or contribute to them. Think of it as a collaborative workspace for writers, but instead of stories or essays, people work on computer programs.

GitHub repository:

https://github.com/enriquea/ZebraQ

Downloading the repo:

$ git clone https://github.com/enriquea/ZebraQ.git

```
eam:~ eam$ git clone https://github.com/enriquea/ZebraQ.git
Cloning into 'ZebraQ'...
remote: Enumerating objects: 75, done.
remote: Counting objects: 100% (75/75), done.
remote: Compressing objects: 100% (61/61), done.
remote: Total 75 (delta 18), reused 61 (delta 11), pack-reused 0
Receiving objects: 100% (75/75), 9.98 MiB | 1.10 MiB/s, done.
Resolving deltas: 100% (18/18), done.
```

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Course materials (Dataset)

Disclaimer: The data used in this course is intended to be used for educational purposes only.

/data folder contains the following files:

- Genes associated with CHD with functional annotations:

  /data/chd_genes.annotations.tsv

- Gene counts from RNA-seq data of wild type and mutant zebrafish (*Danio Rerio*) hearts:

  /data/salmon.merged.gene_counts.tsv

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Part I: Introduction to R

RStudio

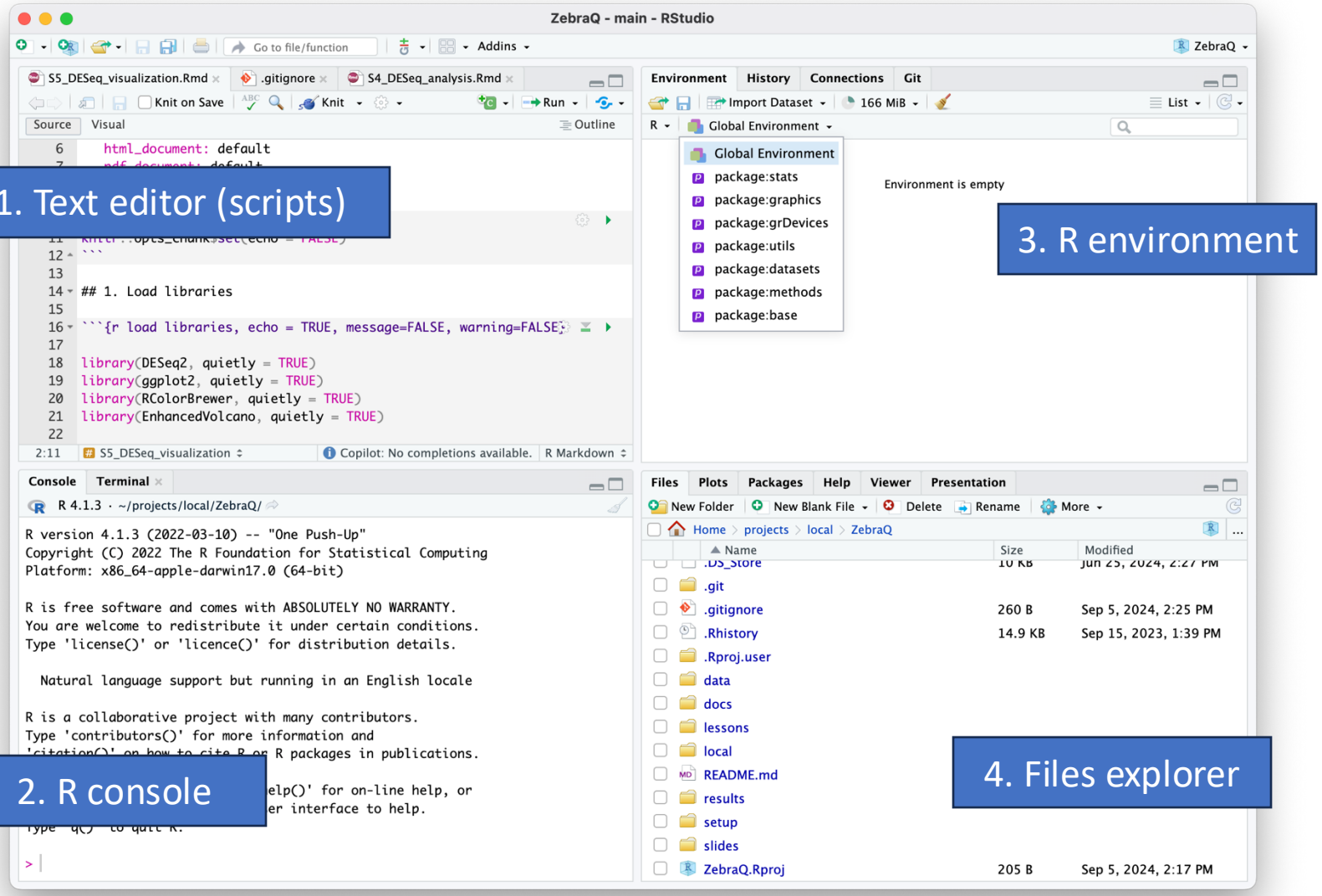

Image credit: https://education.rstudio.com/teach/tools

IDE: *Integrated Development Environment*

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Part I: Introduction to R

Base R
Cheat Sheet

Getting Help

Accessing the help files

?mean
Get help of a particular function.
help.search('weighted mean')
Search the help files for a word or phrase.
help(package = 'dplyr')
Find help for a package.

More about an object

str(iris)
Get a summary of an object's structure.
class(iris)
Find the class an object belongs to.

Using Libraries

install.packages('dplyr')
Download and install a package from CRAN.

library(dplyr)
Load the package into the session, making all its functions available to use.

dplyr::select
Use a particular function from a package.

data(iris)
Load a built-in dataset into the environment.

Working Directory

getwd()
Find the current working directory (where inputs are found and outputs are sent).

setwd('C://file/path')
Change the current working directory.

Use projects in RStudio to set the working directory to the folder you are working in.

Vectors

Creating Vectors

c(2, 4, 6)	2 4 6	Join elements into a vector
2:6	2 3 4 5 6	An integer sequence
seq(2, 3, by=0.5)	2.0 2.5 3.0	A complex sequence
rep(1:2, times=3)	1 2 1 2 1 2	Repeat a vector
rep(1:2, each=3)	1 1 1 2 2 2	Repeat elements of a vector

Vector Functions

sort(x)
Return x sorted.
rev(x)
Return x reversed.
table(x)
See counts of values.
unique(x)
See unique values.

Selecting Vector Elements

By Position

x[4]	The fourth element.
x[-4]	All but the fourth.
x[2:4]	Elements two to four.
x[-(2:4)]	All elements except two to four.
x[c(1, 5)]	Elements one and five.

By Value

x[x == 10]	Elements which are equal to 10.
x[x < 0]	All elements less than zero.
x[x %in% c(1, 2, 5)]	Elements in the set 1, 2, 5.

Named Vectors

x['apple']	Element with name 'apple'.

Programming

For Loop

```
for (variable in sequence){
   Do something
}
```

Example

```
for (i in 1:4){
   j <- i + 10
   print(j)
}
```

While Loop

```
while (condition){
   Do something
}
```

Example

```
while (i < 5){
   print(i)
   i <- i + 1
}
```

If Statements

```
if (condition){
   Do something
} else {
   Do something different
}
```

Example

```
if (i > 3){
   print('Yes')
} else {
   print('No')
}
```

Functions

```
function_name <- function(var){
   Do something
   return(new_variable)
}
```

Example

```
square <- function(x){
   squared <- x*x
   return(squared)
}
```

Reading and Writing Data

Input	Ouput	Description
df <- read.table('file.txt')	write.table(df, 'file.txt')	Read and write a delimited text file.
df <- read.csv('file.csv')	write.csv(df, 'file.csv')	Read and write a comma separated value file. This is a special case of read.table/ write.table.
load('file.RData')	save(df, file = 'file.Rdata')	Read and write an R data file, a file type special for R.

Conditions

a == b	Are equal	a > b	Greater than	a >= b	Greater than or equal to	is.na(a)	Is missing
a != b	Not equal	a < b	Less than	a <= b	Less than or equal to	is.null(a)	Is null

Learn more at **web page or vignette** • package version • Updated: 3/15

https://iqss.github.io/dss-workshops

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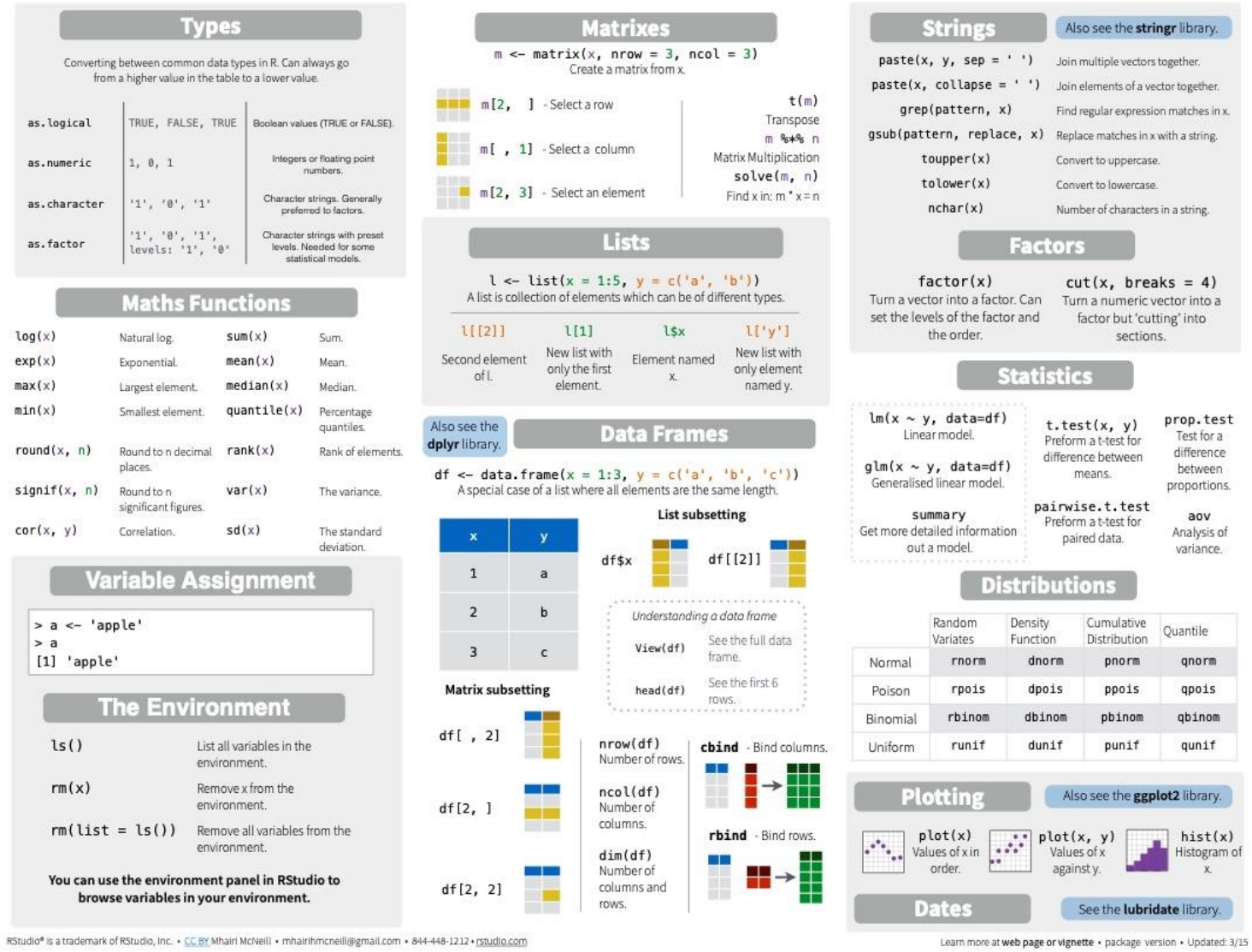
Part I: Introduction to R



Types

Converting between common data types in R. Can always go from a higher value in the table to a lower value.

`as.logical`	`TRUE, FALSE, TRUE`	Boolean values (TRUE or FALSE).
`as.numeric`	`1, 0, 1`	Integers or floating point numbers.
`as.character`	`'1', '0', '1'`	Character strings. Generally preferred to factors.
`as.factor`	`'1', '0', '1', levels: '1', '0'`	Character strings with preset levels. Needed for some statistical models.

Maths Functions

`log(x)`	Natural log.	`sum(x)`	Sum.
`exp(x)`	Exponential.	`mean(x)`	Mean.
`max(x)`	Largest element.	`median(x)`	Median.
`min(x)`	Smallest element.	`quantile(x)`	Percentage quantiles.
`round(x, n)`	Round to n decimal places.	`rank(x)`	Rank of elements.
`signif(x, n)`	Round to n significant figures.	`var(x)`	The variance.
`cor(x, y)`	Correlation.	`sd(x)`	The standard deviation.

Variable Assignment

```
> a <- 'apple'
> a
[1] 'apple'
```

The Environment

`ls()`	List all variables in the environment.
`rm(x)`	Remove x from the environment.
`rm(list = ls())`	Remove all variables from the environment.

You can use the environment panel in RStudio to browse variables in your environment.

Matrixes

`m <- matrix(x, nrow = 3, ncol = 3)`
Create a matrix from x.

`m[2, ]` - Select a row
`m[ , 1]` - Select a column
`m[2, 3]` - Select an element

`t(m)` Transpose
`m %*% n` Matrix Multiplication
`solve(m, n)` Find x in: m * x = n

Lists

`l <- list(x = 1:5, y = c('a', 'b'))`
A list is collection of elements which can be of different types.

`l[[2]]`	`l[1]`	`l$x`	`l['y']`
Second element of l.	New list with only the first element.	Element named x.	New list with only element named y.

Also see the **dplyr** library.

Data Frames

`df <- data.frame(x = 1:3, y = c('a', 'b', 'c'))`
A special case of a list where all elements are the same length.

x	y
1	a
2	b
3	c

List subsetting

`df$x` `df[[2]]`

Understanding a data frame

`View(df)` See the full data frame.
`head(df)` See the first 6 rows.

Matrix subsetting

`df[ , 2]`
`df[2, ]`
`df[2, 2]`

`nrow(df)` Number of rows.
`ncol(df)` Number of columns.
`dim(df)` Number of columns and rows.

`cbind` - Bind columns.
`rbind` - Bind rows.

Strings

Also see the **stringr** library.

`paste(x, y, sep = ' ')`	Join multiple vectors together.
`paste(x, collapse = ' ')`	Join elements of a vector together.
`grep(pattern, x)`	Find regular expression matches in x.
`gsub(pattern, replace, x)`	Replace matches in x with a string.
`toupper(x)`	Convert to uppercase.
`tolower(x)`	Convert to lowercase.
`nchar(x)`	Number of characters in a string.

Factors

`factor(x)` Turn a vector into a factor. Can set the levels of the factor and the order.

`cut(x, breaks = 4)` Turn a numeric vector into a factor but 'cutting' into sections.

Statistics

`lm(x ~ y, data=df)` Linear model.
`glm(x ~ y, data=df)` Generalised linear model.
`summary` Get more detailed information out a model.

`t.test(x, y)` Preform a t-test for difference between means.
`pairwise.t.test` Preform a t-test for paired data.

`prop.test` Test for a difference between proportions.
`aov` Analysis of variance.

Distributions

	Random Variates	Density Function	Cumulative Distribution	Quantile
Normal	`rnorm`	`dnorm`	`pnorm`	`qnorm`
Poison	`rpois`	`dpois`	`ppois`	`qpois`
Binomial	`rbinom`	`dbinom`	`pbinom`	`qbinom`
Uniform	`runif`	`dunif`	`punif`	`qunif`

Plotting

Also see the **ggplot2** library.

`plot(x)` Values of x in order.
`plot(x, y)` Values of x against y.
`hist(x)` Histogram of x.

Dates

See the **lubridate** library.

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https://iqss.github.io/dss-workshops

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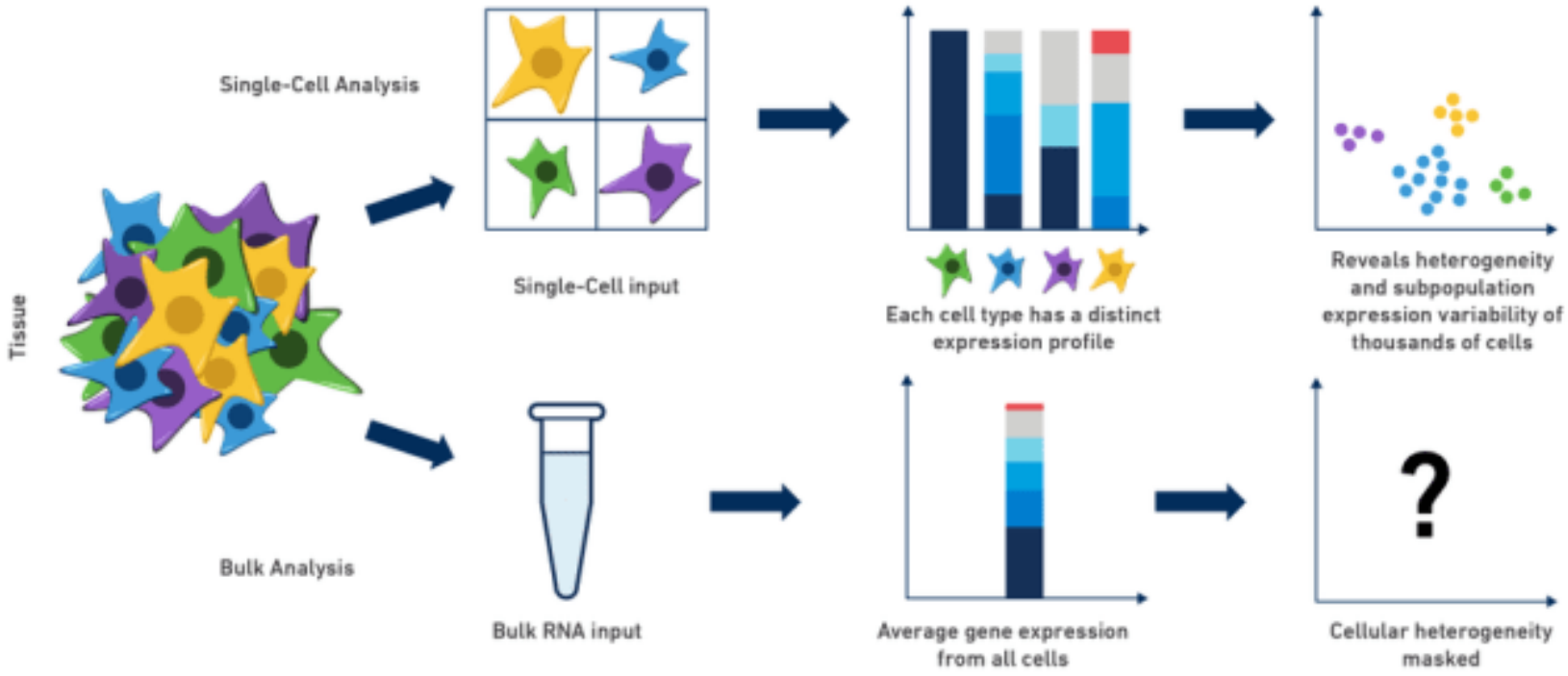
Bulk vs scRNA-seq



https://www.10xgenomics.com/blog/single-cell-rna-seq-an-introductory-overview-and-tools-for-getting-started

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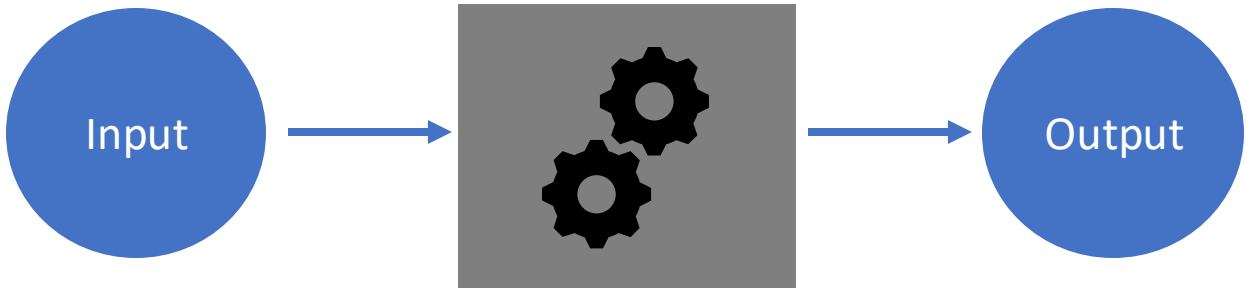
RNAseq pipeline



Black box?

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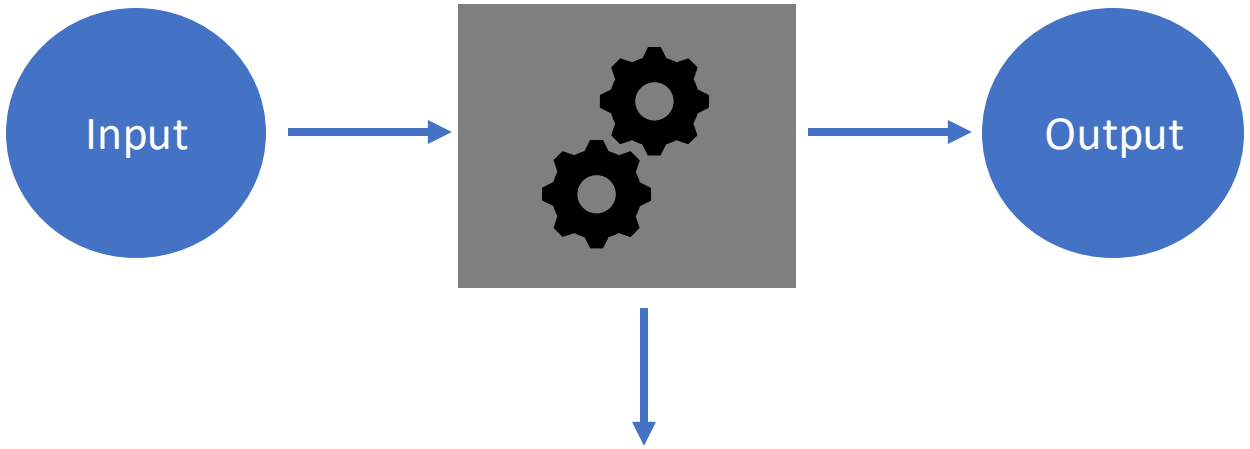
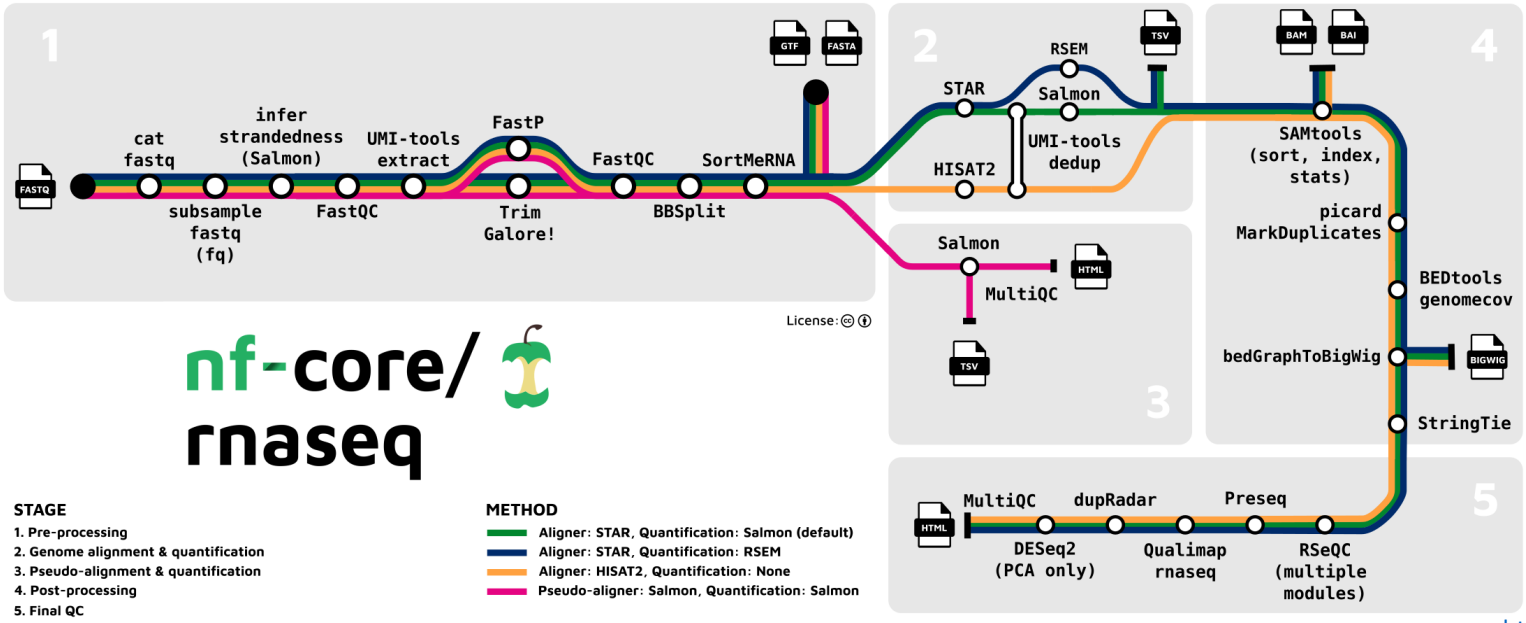
RNAseq pipeline





https://nf-co.re/rnaseq

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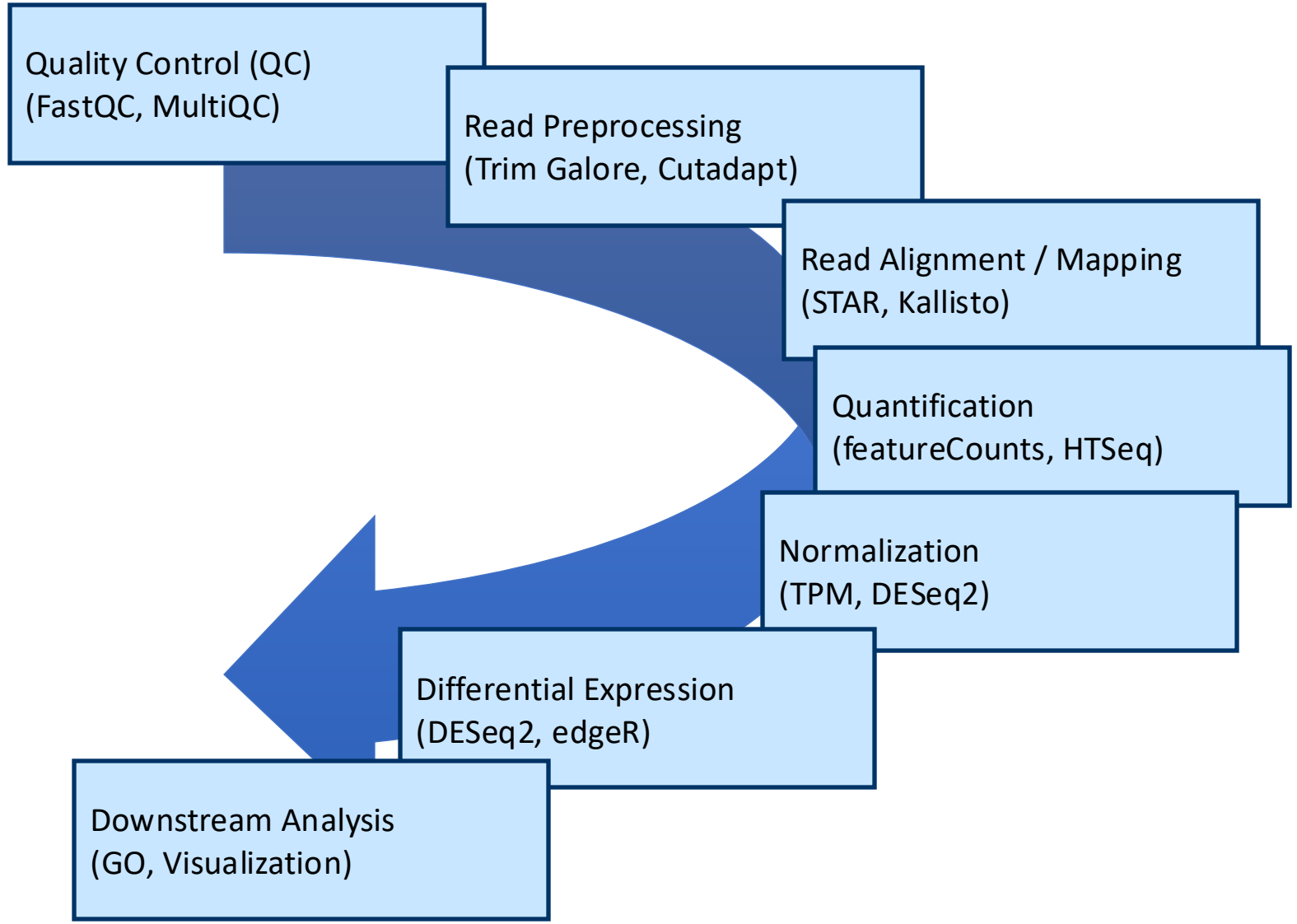
RNA-seq pipeline: main bioinformatics steps



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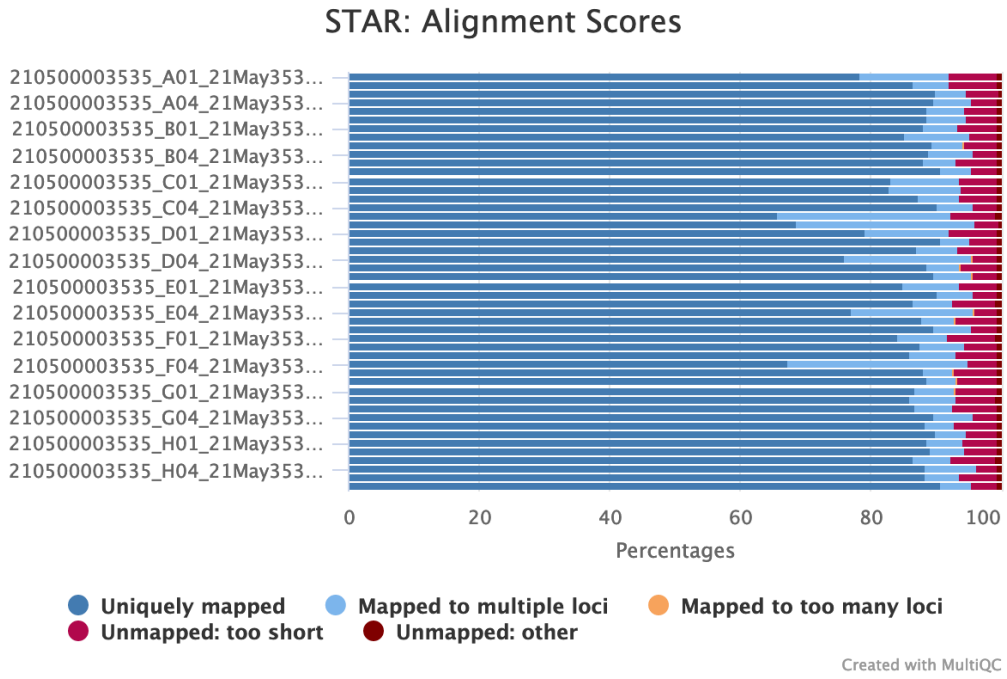
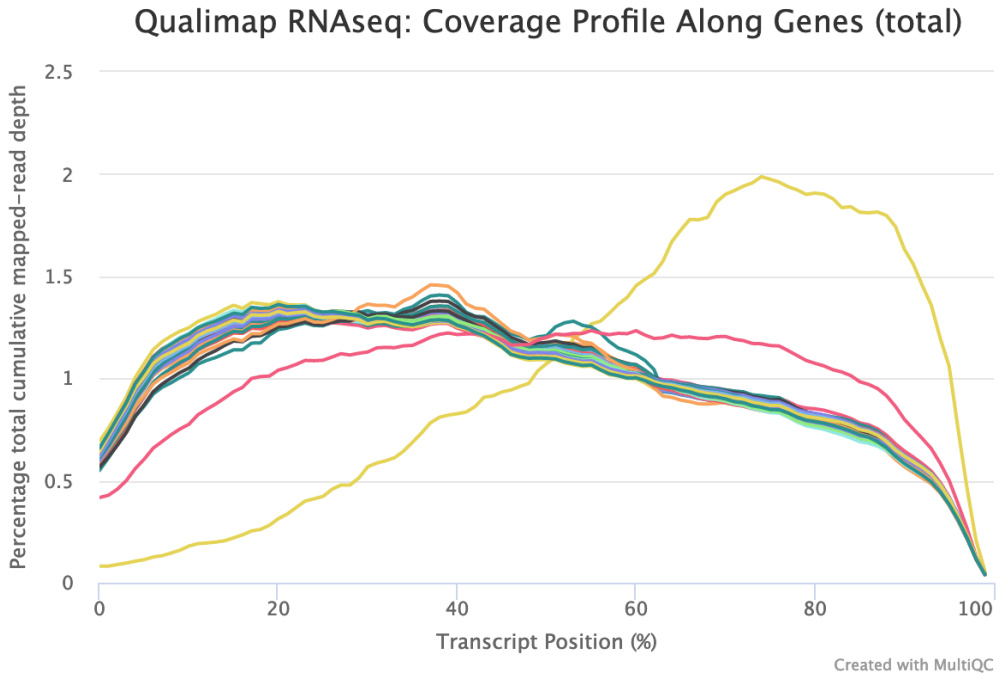
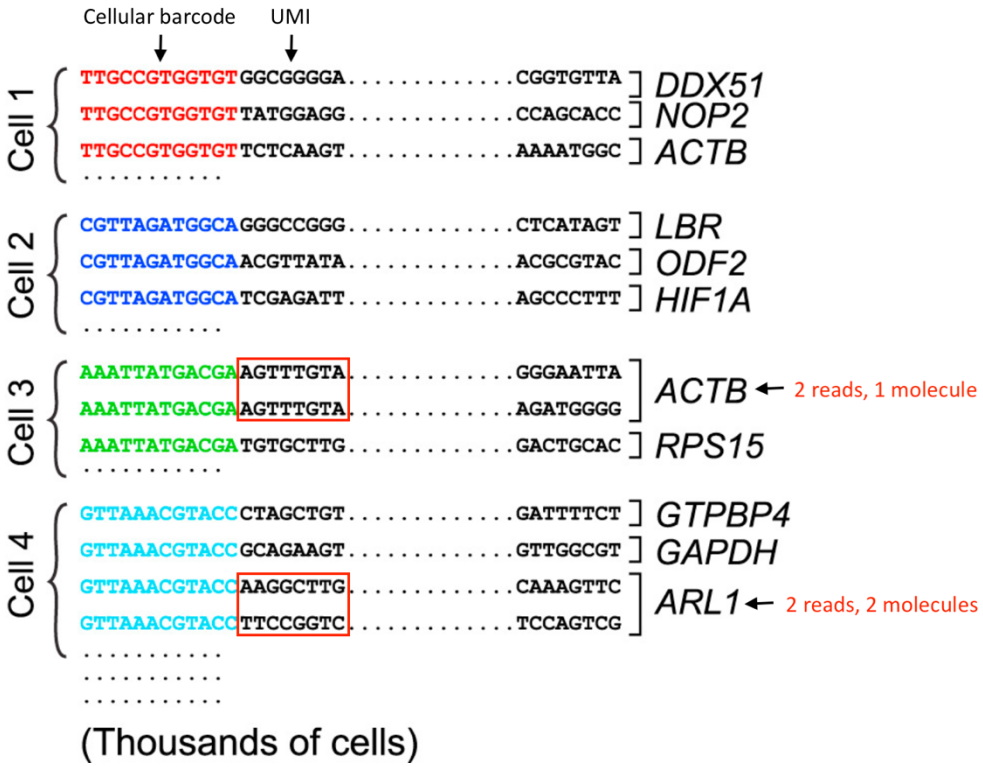
QC visualization and monitoring





“Matrix count”

“The outcome of this procedure is a gene/cell count matrix, which is used as an estimate of the number of RNA molecules in each cell for each gene”



	Cell1	Cell2	…	CellN
Gene1	3	2	.	13
Gene2	2	3	.	1
Gene3	1	14	.	18
…	.	.	.	.
…	.	.	.	.
…	.	.	.	.
GeneM	25	0	.	0

Source: https://github.com/hbctraining/scRNA-seq

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Results from DESeq experiment

The results table when printed will provide the information about the comparison, e.g. "log2 fold change (MAP): condition treated vs untreated", meaning that the estimates are of log2(treated / untreated).

```
[1] "Comparision performed: condition_MT_vs_WT"
log2 fold change (MLE): condition MT vs WT
Wald test p-value: condition MT vs WT
DataFrame with 13755 rows and 6 columns
               baseMean log2FoldChange     lfcSE       stat      pvalue        padj
              <numeric>      <numeric> <numeric>  <numeric>   <numeric>   <numeric>
LOC100000024   35.01346      1.2466174  0.670687  1.8587185    0.063067    0.125215
LOC100000576  401.25055     -0.2115917  0.220422 -0.9599394    0.337086    0.467804
LOC100000851    2.21568      0.0301926  1.226557  0.0246157    0.980361    0.988411
LOC100001344   61.36695     -0.1027153  0.246557 -0.4165978    0.676973    0.774125
LOC100001550   42.24091      0.5415869  0.334623  1.6184984    0.105555    0.190337
...                 ...            ...       ...        ...         ...         ...
zwilch        400.60805      0.5873992  0.121817   4.821984 1.42137e-06 1.21568e-05
zyg11        1465.05339     -0.5332838  0.168022  -3.173889 1.50411e-03 5.47396e-03
zymnd12         8.45217     -0.6658308  0.683108  -0.974708 3.29705e-01 4.60536e-01
zyx          1029.10390     -0.0871254  0.148073  -0.588395 5.56267e-01 6.73980e-01
zzz3         1124.36740     -0.4385312  0.207517  -2.113229 3.45811e-02 7.64093e-02
```

Log2FoldChange: For a given comparison, a positive fold change value indicates an increase of expression, while a negative fold change indicates a decrease in expression.

P-value: Indicates whether the gene analysed is likely to be differentially expressed in that comparison.

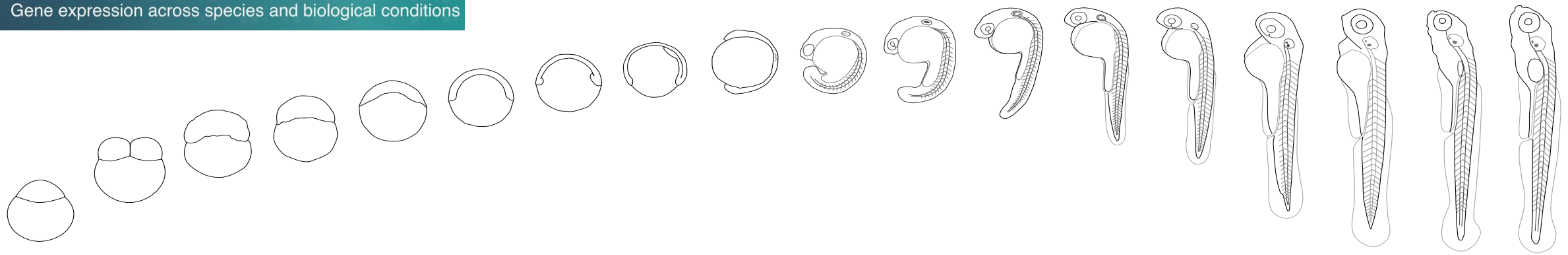
Adj. p-value: The p-value obtained for each gene above is re-calculated to correct for multiple testing (n genes).

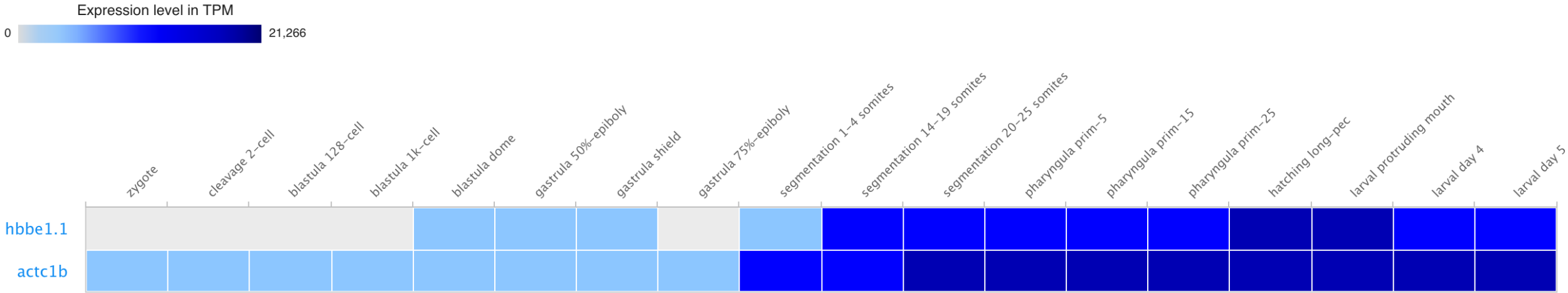
https://biocorecrg.github.io/CRG_Bioinformatics_for_Biologists/differential_gene_expression.html

Comparing results with external resources/databases

Expression Atlas

Gene expression across species and biological conditions





https://www.ebi.ac.uk/gxa/experiments/E-ERAD-475/Results; https://doi.org/10.7554/eLife.30860

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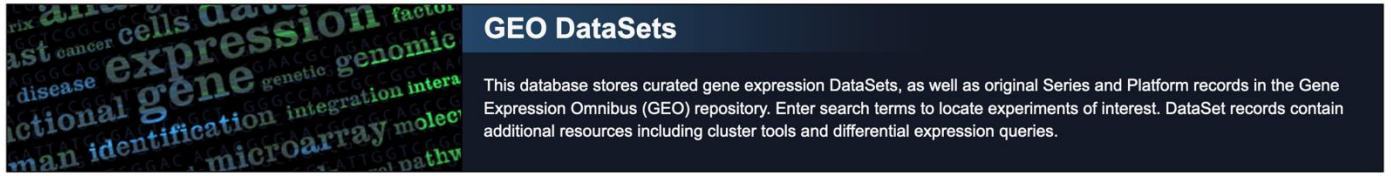
Other resources & databases

- Co-expression analysis



https://www.ncbi.nlm.nih.gov/geo/

- Protein-protein interaction analysis

STRING https://string-db.org/

- Pathway enrichment analysis

reactome https://reactome.org/

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