Bioinformatics Summer 2020

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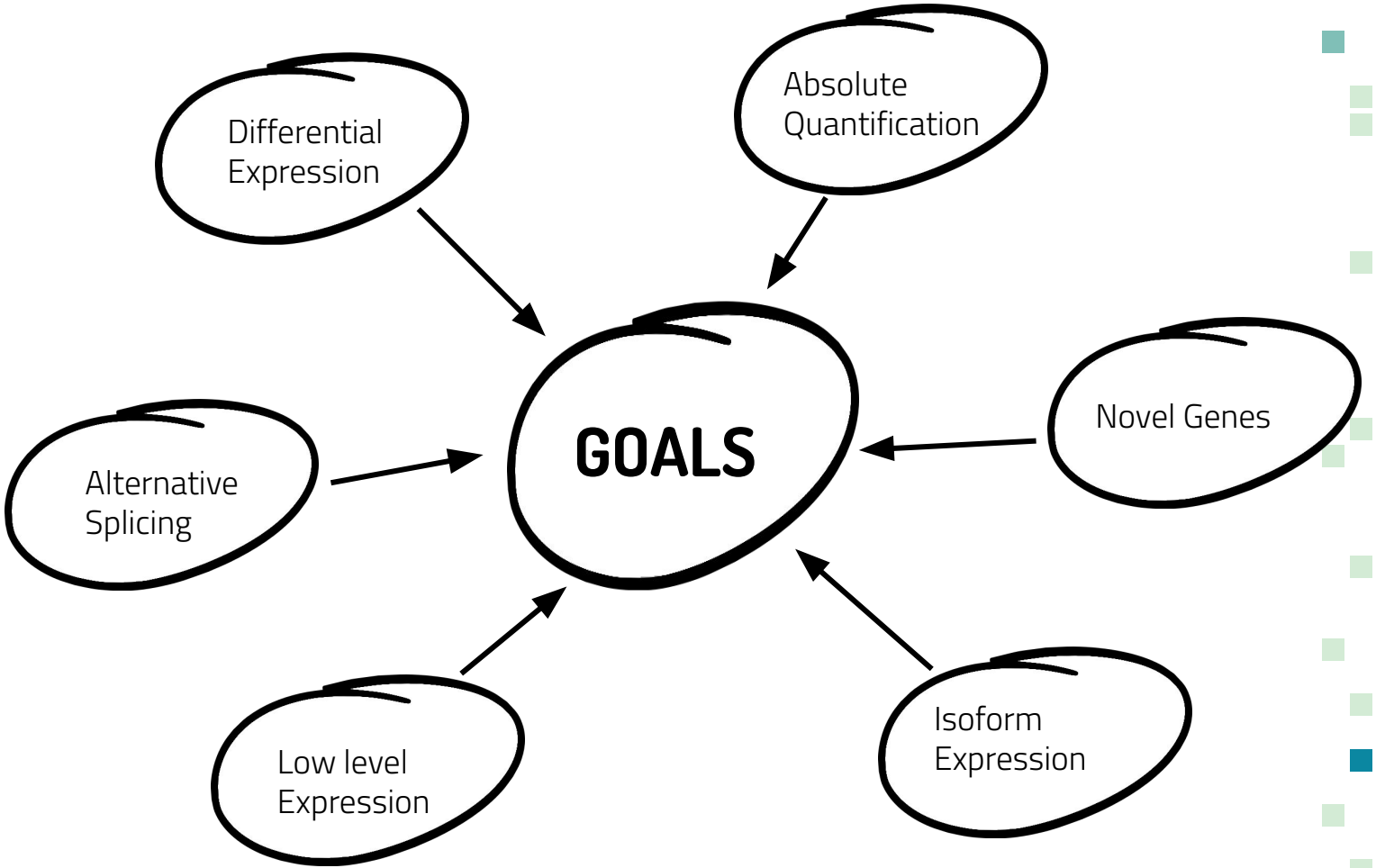
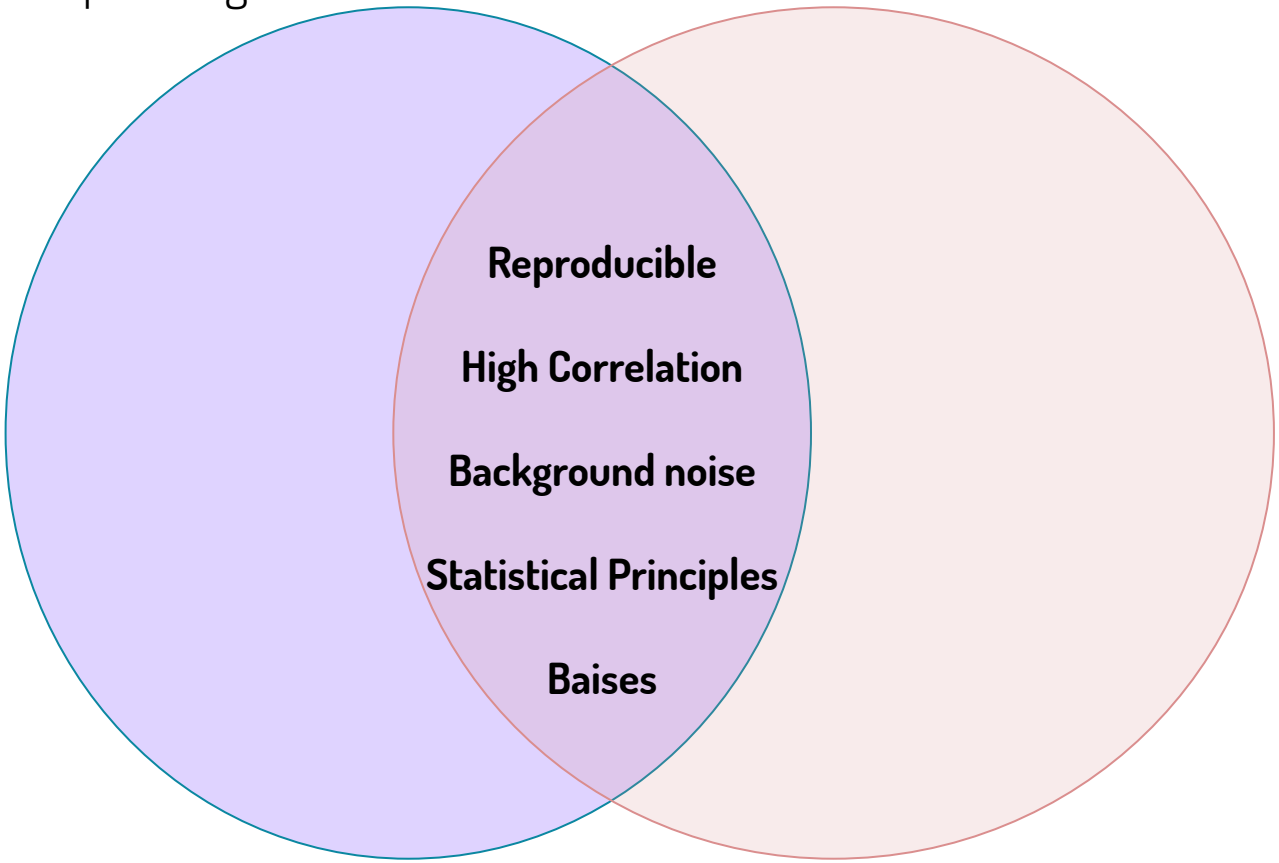
Microarrays vs RNA sequencing

Practical Questions

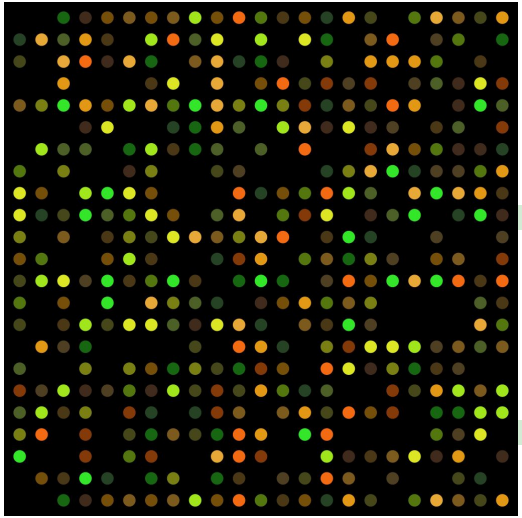
- What genome information is available for my species of interest?
- How much data analysis expertise do I have or have access to?
- How much money do I have to spend?


Differential Expression
Absolute Quantification
Alternative Splicing
GOALS
Novel Genes
Low level Expression
Isoform Expression


RNA Sequencing
Microarray
Reproducible
High Correlation
Background noise
Statistical Principles
Baises

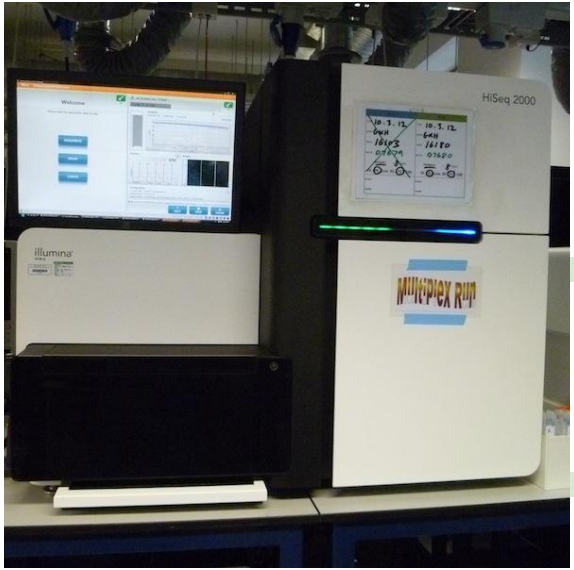
Microarrays

- Robust, reliable method, proven over decades of use
- High throughput method - 1000s of samples analysed per month
- Streamlined handling - can easily be automated
- Straightforward data analysis
- Low cost
- Dependent on prior sequence knowledge
- Cannot detect structural variation
- Cannot detect isoforms

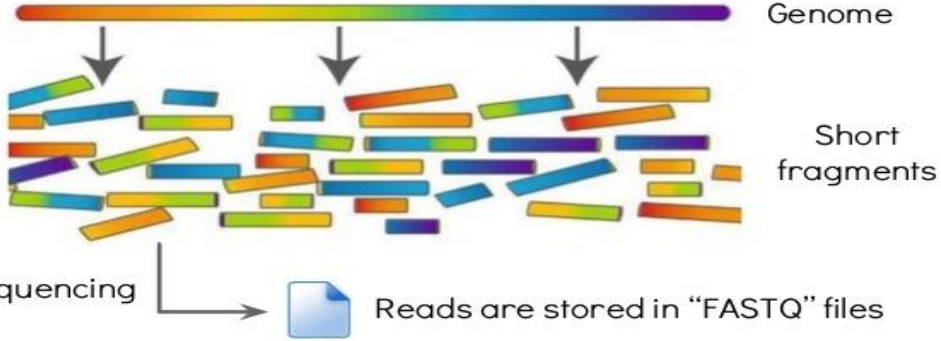


RNA Sequencing

- Provides a comprehensive view of the transcriptome
- Not dependent on any prior sequence knowledge
- Can detect structural variations such as gene fusion and alternative splicing events
- A truly digital solution (absolute abundance vs relative abundance)
- Data storage is more challenging
- Analysis is more complex - no standard protocol
- Specialized computing infrastructure and personnel is required
- More expensive than microarrays



An in-depth look to sequences and RNAseq pipeline



Sequence ID

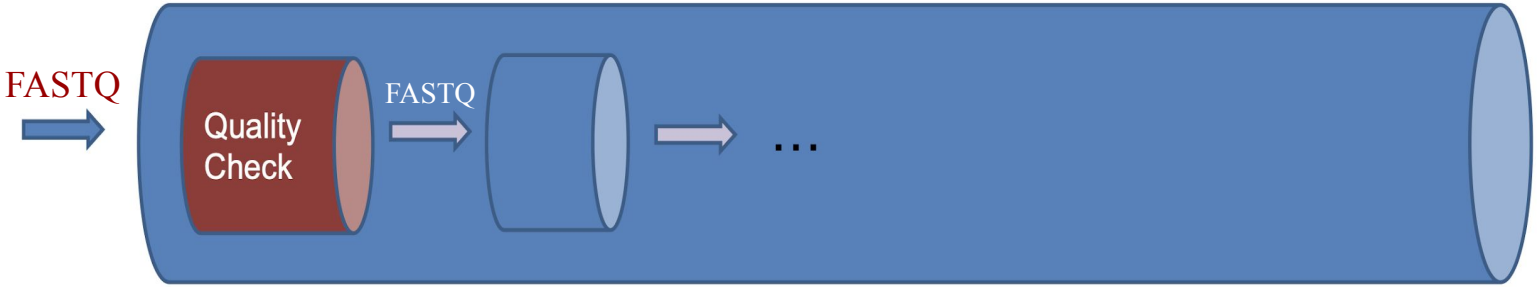
Sequenced Read

```
@SRR081708.237649/1
GGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGG
+
[illegible]>:<9>>>:=;>>?<>:@?>;==@@@>?=AAA<>=A@?6>4B=<>>.@>?<@;?#############
```

Blank

Quality Score

High Level Data Flow Management



The typical first step of a NGS analysis pipeline is the quality check of produced sequences.

FASTQC: free program that reports quality profile of reads

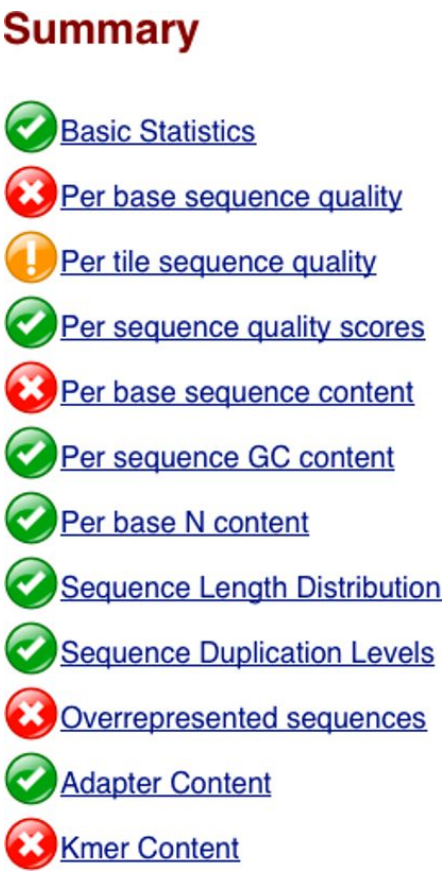
http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

1) Run FASTQC

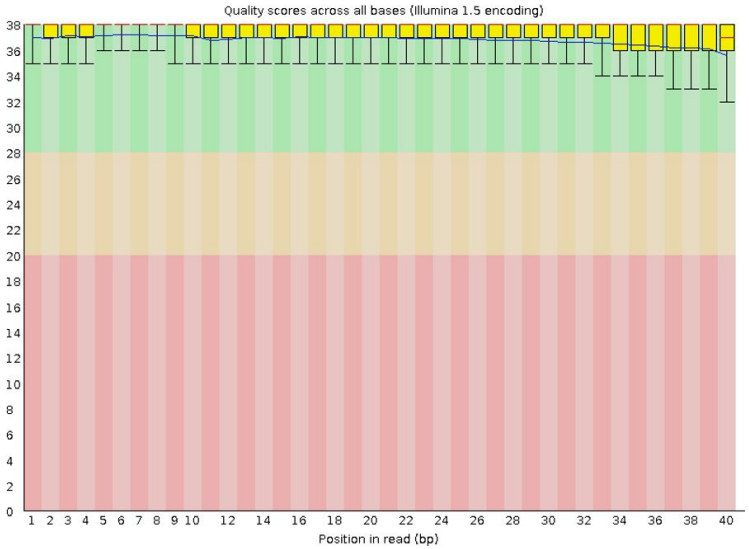
fastqc sample.fastq

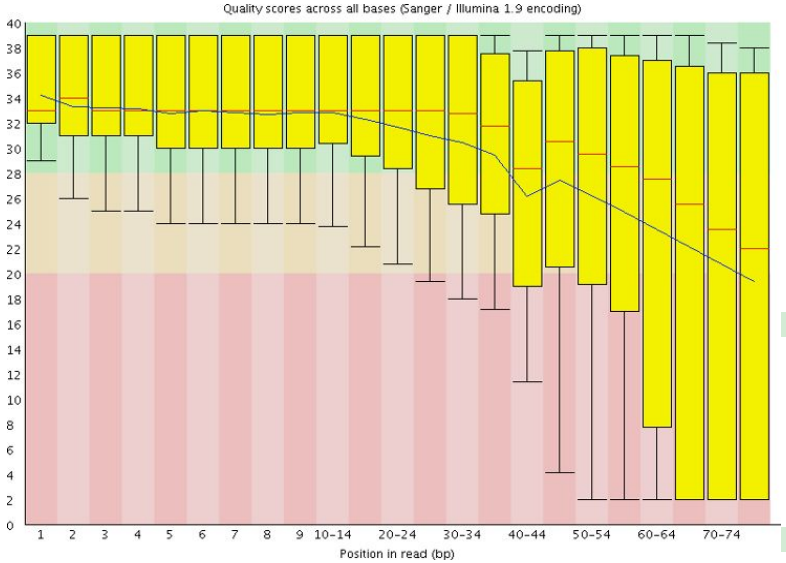
2) Open the output file

sample_fastq.html

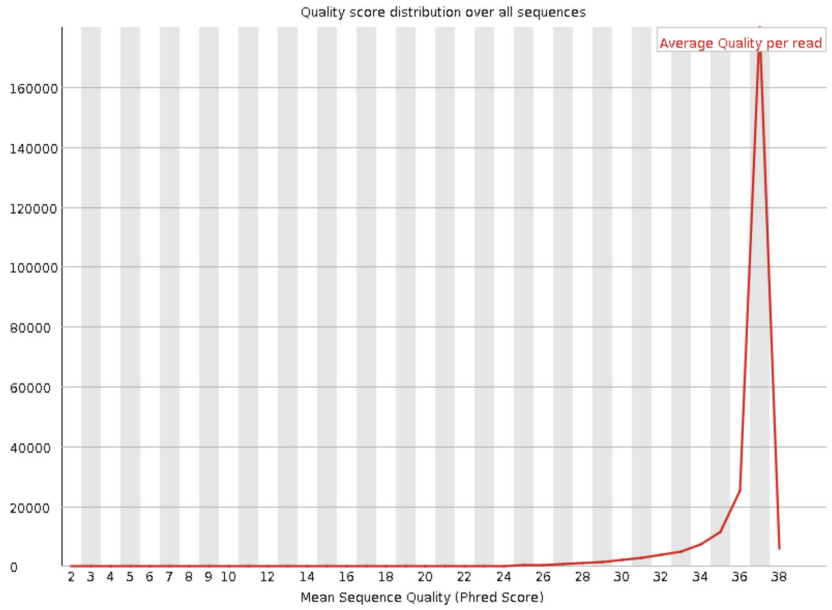


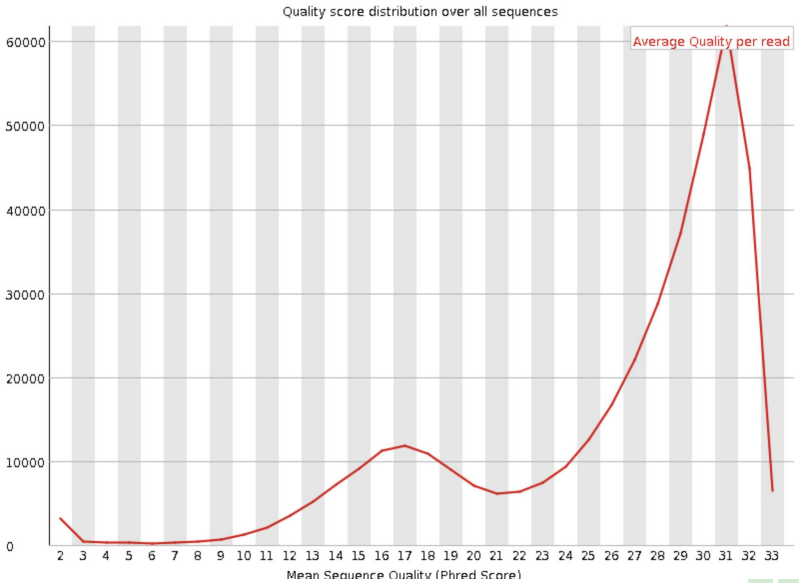
FASTQC: Per base sequence quality



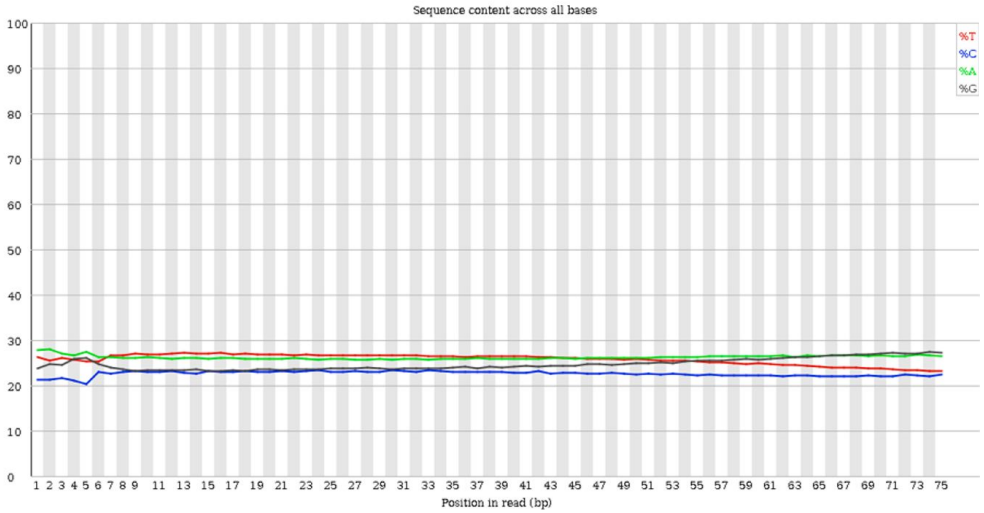


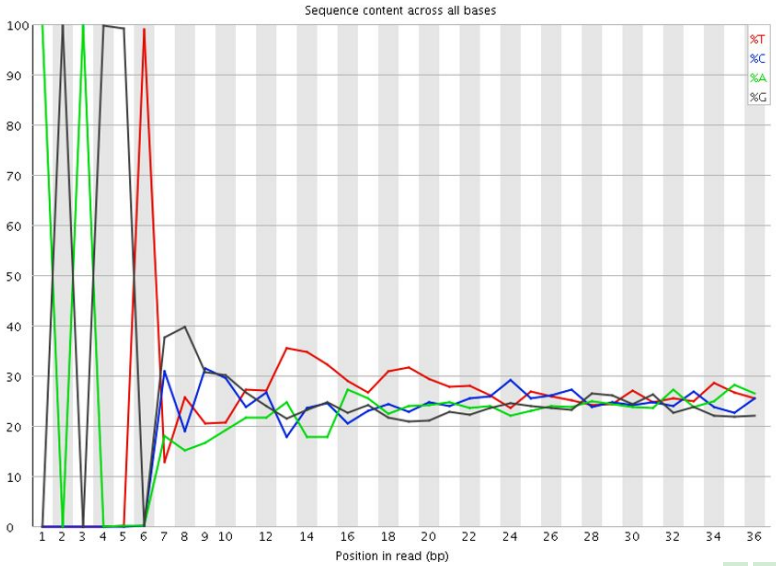
FASTQC: Per sequence quality scores



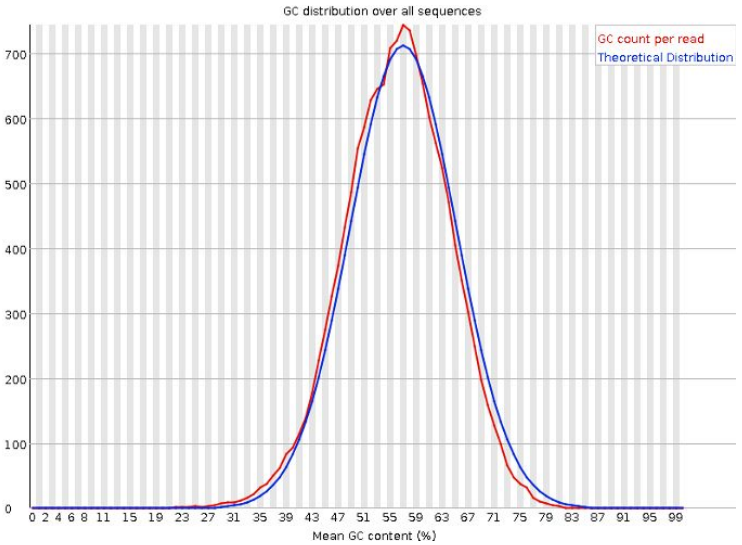


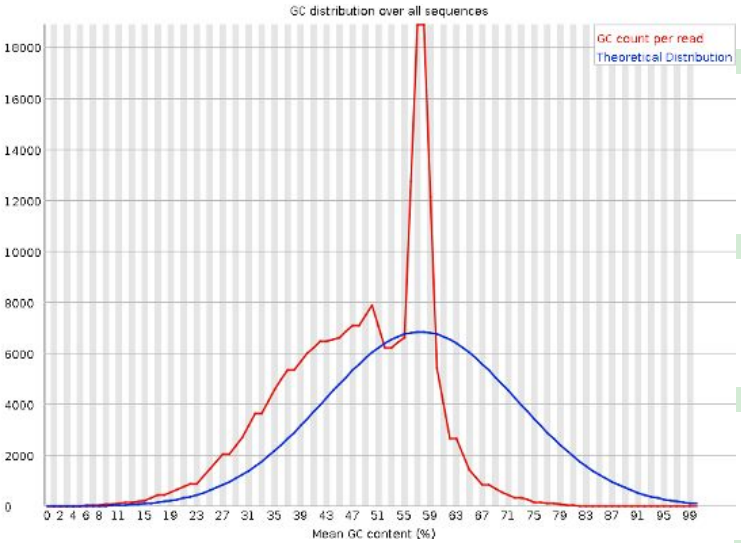
FASTQC: Per base sequence content



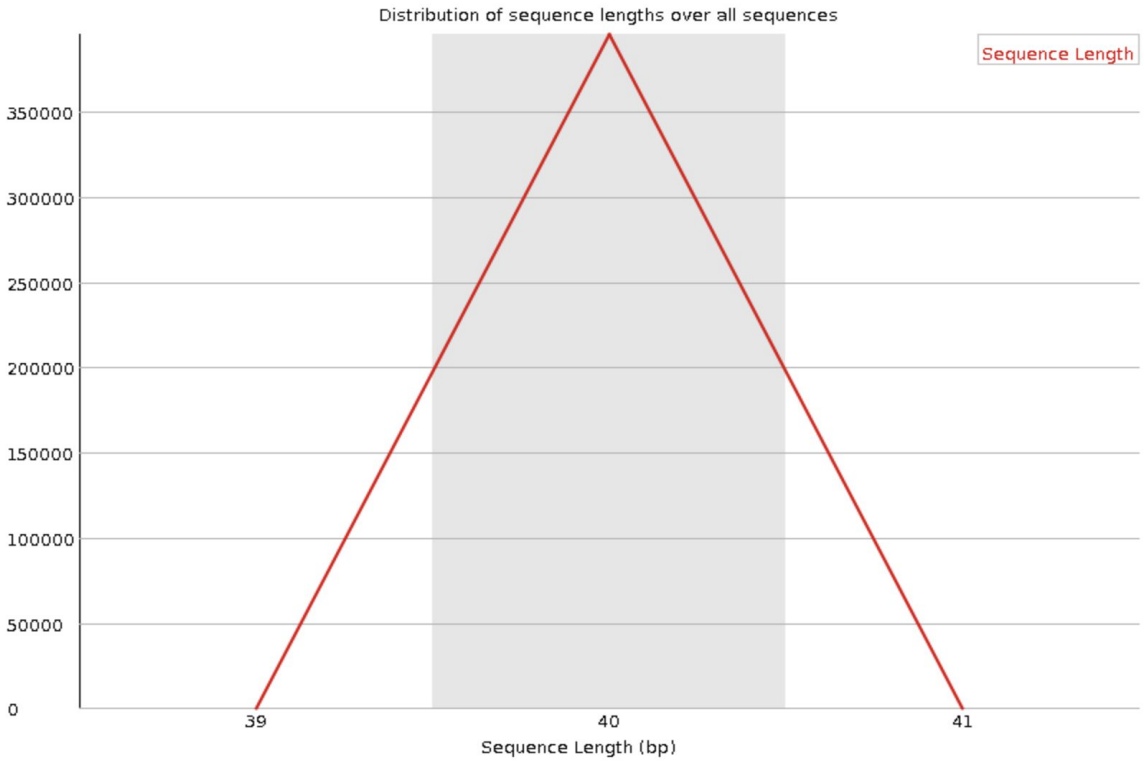


FASTQC: Per sequence GC content

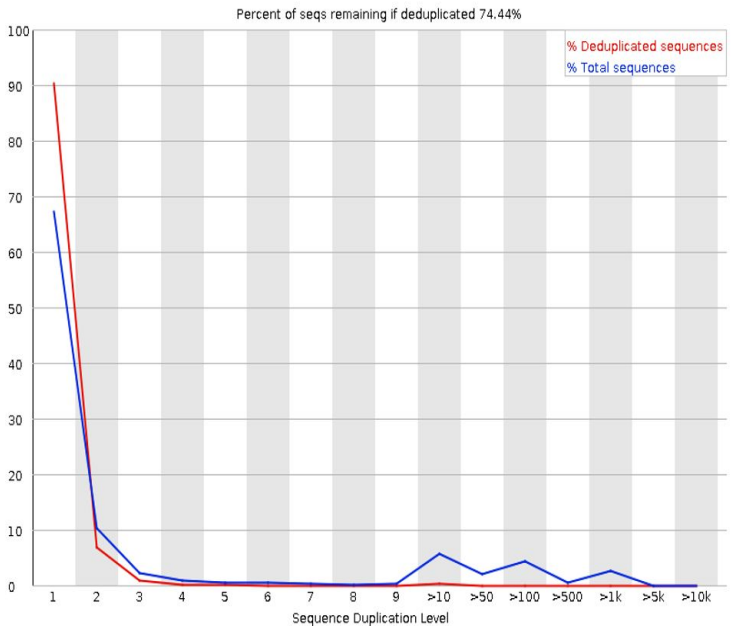


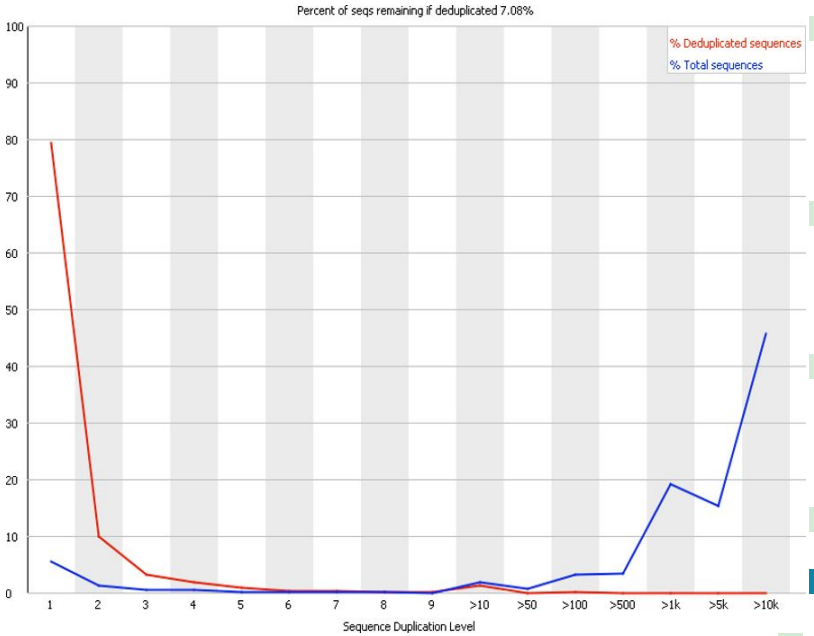


FASTQC: Sequence Length Distribution

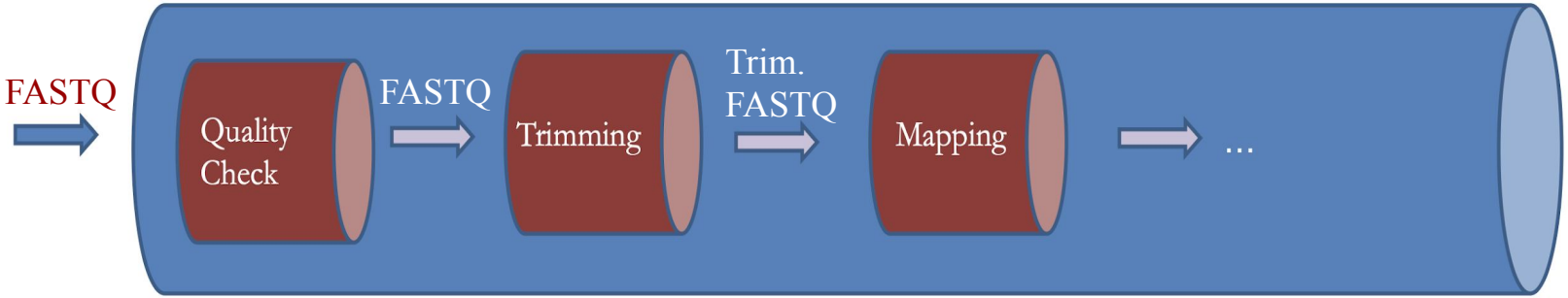


FASTQC: Sequence duplication levels



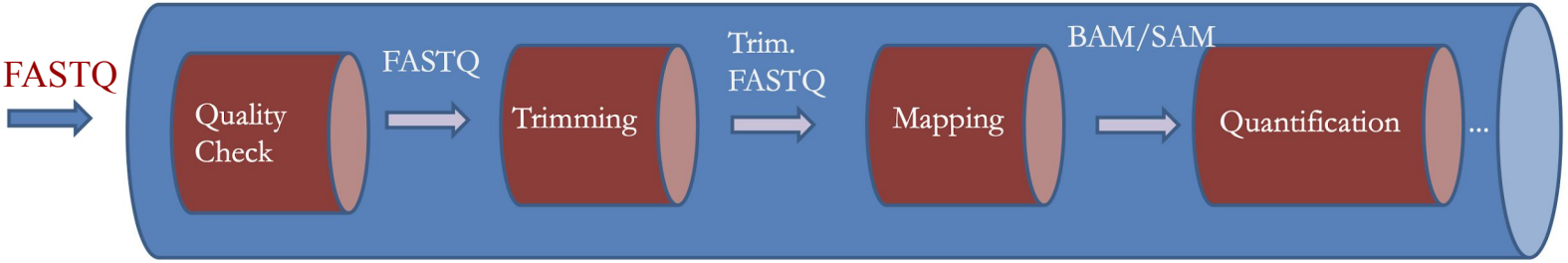


Another step into our pipeline...



Read Mapping

Almost at the end of the tunnel!

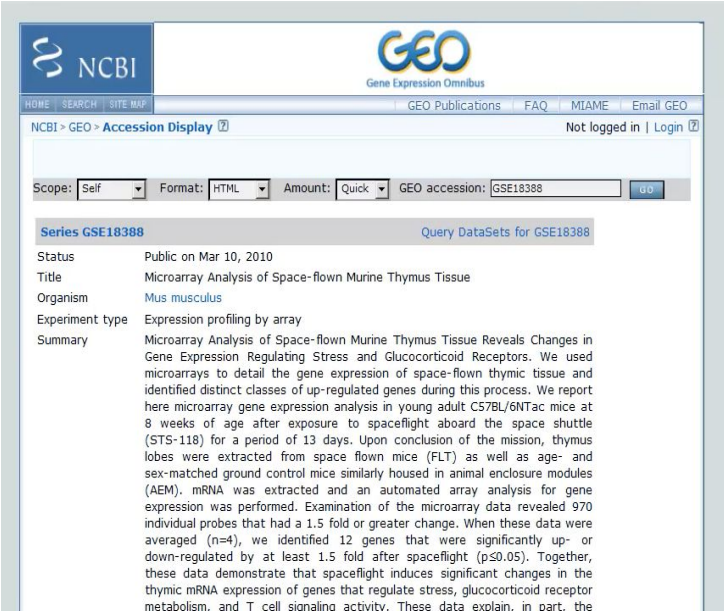


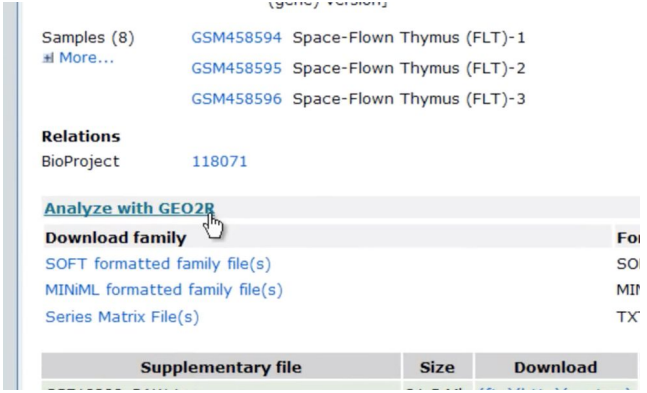
Different Measures:

- Raw counts
- Counts per million reads (CPMR)
- RPKM
- FPKM
- Transcripts per million

GEO2R

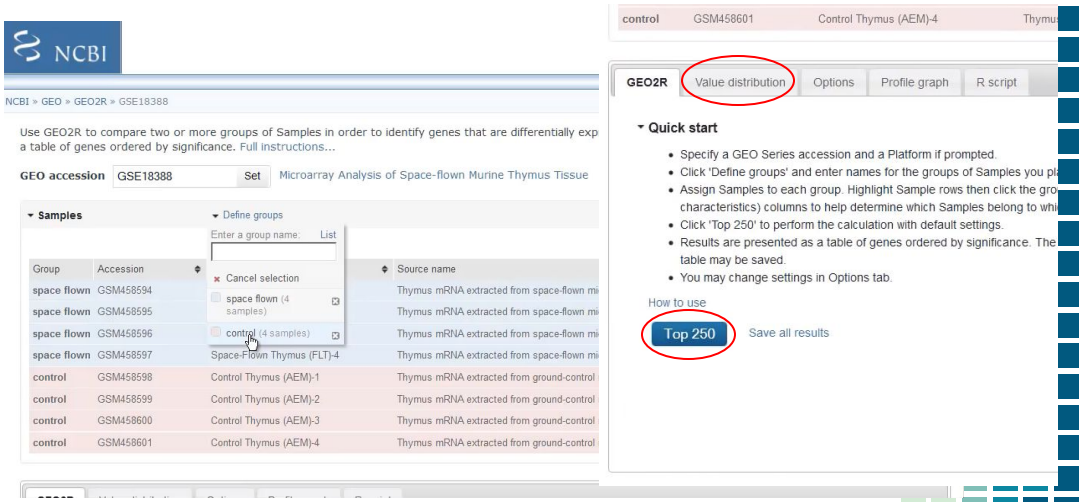
- Interactive web tool
- Can compare 2+ groups of samples in the GEO series
- **Goal: Identify genes that are differentially expressed across experimental conditions**
- Greater proportion of GEO data can be analyzed in a more time-efficient manner





GEO2R

- “Define Groups” for your samples
- Value of distributions
- Table of results ordered by significance (**lower P value = more significant**)



ID	adj.P.Val	P.Value	t	B	logFC	Gene.symbol	Gene.title
10358454	0.00509	1.96e-07	-13.48	4.3773	-1.384	Rbm3	RNA binding motif pr...
10603469	0.00509	2.86e-07	-12.92	4.24228	-1.304	Rbm3	RNA binding motif pr...
10556113	0.00546	4.61e-07	-12.24	4.06259	-1.408	Rbm3	RNA binding motif pr...
10535904	0.00844	9.50e-07	11.28	3.76621	1.894	Hsph1	heat shock 105kDa/1...
10490946	0.03472	5.44e-06	9.21	2.93118	1.02	Hsp90aa1	heat shock protein 9...
10514713	0.03472	5.86e-06	9.13	2.89184	0.948	Wdr78	WD repeat domain 78
10531415	0.03973	7.82e-06	8.83	2.73562	1.499	Cxcl10	chemokine (C-X-C m...
10391146	0.04368	9.83e-06	8.59	2.60896	1.232	Acly	ATP citrate lyase
10497079	0.0521	1.32e-05	8.29	2.44138	1.251	Ptger3	prostaglandin E rece...
10378848	0.05835	1.64e-05	8.08	2.31365	0.882	Hsp90aa1	heat shock protein 9...
10402615	0.07332	2.27e-05	7.77	2.11964	0.853	Hsp90aa1	heat shock protein 9...
10465604	0.11029	3.72e-05	7.31	1.81172	0.881	Stip1	stress-induced phos...
10548194	0.11323	4.14e-05	7.21	1.74391	0.933	Fkbp4	FK506 binding protei...
10343263	0.12007	4.73e-05	-7.09	1.65832	-1.149		
10475502	0.12943	5.50e-05	-6.96	1.55967	-1.182		
10565794	0.12943	5.82e-05	6.91	1.52204	0.869	Serpinh1	serine (or cysteine) p...
10413542	0.13514	6.46e-05	6.82	1.45337	0.749	Tkt	transketolase
10342984	0.17386	8.82e-05	-6.56	1.24409	-1.035		
10580382	0.17386	9.82e-05	6.47	1.17061	0.695	Neto2	neuropilin (NRP) and...
10517727	0.17386	1.02e-04	6.44	1.14785	0.757	Klhdc7a	kelch domain contai...
10584578	0.17386	1.03e-04	6.42	1.13682	1.008	Hspa8	heat shock protein 8
10565609	0.17386	1.08e-04	6.39	1.10817	1.211	Thrsp	thyroid hormone resp...
10481111	0.18898	1.22e-04	6.29	1.01954	0.761		
10364712	0.19171	1.29e-04	-6.24	0.97985	-1.268	Cirbp	cold inducible RNA b...

R Overview

- R is a language and environment for statistical computing and graphics.
- Includes an effective data handling and storage facility
- Includes graphical facilities for data analysis and display

R - Vector

```
v <- c(1,2,3,4) ## Create a vector v with element 1, 2. 3, 4

v[3]            # access 3rd element
v[c(2, 4)]      # access 2nd and 4th element
v[-1]           # access all but 1st element

v[2] <- 0       # modify 2nd element
v[x<3] <- 5     # modify elements less than 0
v <- x[1:4]     # truncate x to first 4 elements
```

R- Matrix (Creation)

```
> A=matrix(1:9, nrow = 3, ncol = 3) ##Create a 3 by 3 matrix
> A
     [,1] [,2] [,3]
[1,]    1    4    7
[2,]    2    5    8
[3,]    3    6    9
```

```
> cbind(c(1,2,3),c(4,5,6))
[,1] [,2]
[1,]      1      4
[2,]      2      5
[3,]      3      6
> rbind(c(1,2,3),c(4,5,6))
[,1] [,2] [,3]
[1,]      1      2      3
[2,]      4      5      6
```

R- Matrix (vector as index)

```
> x
[,1] [,2] [,3]
[1,]     1     4     7
[2,]     2     5     8
[3,]     3     6     9
> x[c(1,2),c(2,3)]     # select rows 1 & 2 and columns 2 & 3
[,1] [,2]
[1,]     4     7
[2,]     5     8
> x[c(3,2),]     # leaving column field blank will select entire columns
[,1] [,2] [,3]
[1,]     3     6     9
[2,]     2     5     8
> x[,]     # leaving row as well as column field blank will select entire matrix
[,1] [,2] [,3]
[1,]     1     4     7
[2,]     2     5     8
[3,]     3     6     9
> x[-1,]     # select all rows except first
[,1] [,2] [,3]
[1,]     2     5     8
[2,]     3     6     9
```

Volcano Plot

- Volcano plots represent a useful way to visualize the results of differential expression analyses.

- A volcano plot is a type of scatterplot that shows statistical significance (P value) versus magnitude of change (fold change).

Volcano Plot (Installation and Syntax)

```
BiocManager::install('EnhancedVolcano')
library(EnhancedVolcano)
```

```
EnhancedVolcano(data, lab = rownames(data),
x = 'log2FoldChange', y = 'pvalue', xlim = c(..., ...))
```

Example: Volcano Plot (Data Input)

ID	adj.P.Val	P.Value	t	B	logFC	Gene.symbol	Gene.title
10358454	0.00509	1.96e-07	-13.48	4.3773	-1.384	Rbm3	RNA binding motif pr...
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10490946	0.03472	5.44e-06	9.21	2.93118	1.02	Hsp90aa1	heat shock protein 9...
10514713	0.03472	5.86e-06	9.13	2.89184	0.948	Wdr78	WD repeat domain 78
10531415	0.03973	7.82e-06	8.83	2.73562	1.499	Cxcl10	chemokine (C-X-C m...
10391146	0.04368	9.83e-06	8.59	2.60896	1.232	Acly	ATP citrate lyase
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10402615	0.07332	2.27e-05	7.77	2.11964	0.853	Hsp90aa1	heat shock protein 9...
10465604	0.11029	3.72e-05	7.31	1.81172	0.881	Stip1	stress-induced phos...
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10343263	0.12007	4.73e-05	-7.09	1.65832	-1.149		
10475502	0.12943	5.50e-05	-6.96	1.55967	-1.182		
10565794	0.12943	5.82e-05	6.91	1.52204	0.869	Serpinh1	serine (or cysteine) p...
10413542	0.13514	6.46e-05	6.82	1.45337	0.749	Tkt	transketolase
10342984	0.17386	8.82e-05	-6.56	1.24409	-1.035		
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10517727	0.17386	1.02e-04	6.44	1.14785	0.757	Klhdc7a	kelch domain contai...
10584578	0.17386	1.03e-04	6.42	1.13682	1.008	Hspa8	heat shock protein 8
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10364712	0.19171	1.29e-04	-6.24	0.97965	-1.268	Cirbp	cold inducible RNA b...

Example: Volcano Plot (Creation)

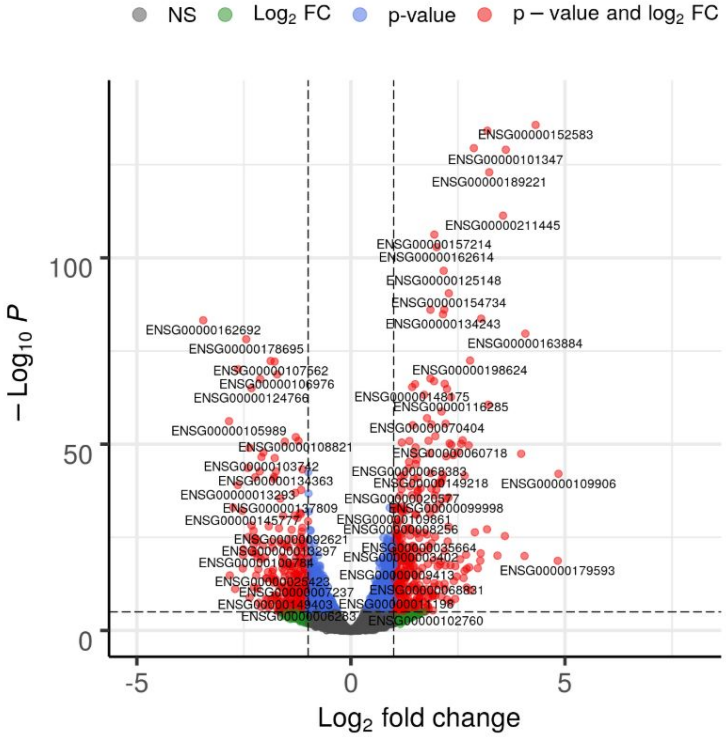
```
EnhancedVolcano(data, lab = rownames(data),
 x = 'log2FoldChange', y = 'pvalue', xlim = c(-5, 5))
```

Volcano Plot (Full Code)

```
# install package
BiocManager::install("EnhancedVolcano")
library(EnhancedVolcano)

# load data
genetable <- read.table("geo2r.txt", header = T)
# use the ID to name the rowname
rownames(genetable) <- genetable$ID
# make plot and modify cut-offs for log2FC and P value
# ; specify title; adjust point and label size
EnhancedVolcano_plot<- EnhancedVolcano(genetable,
                 lab = rownames(genetable),
                 pCutoff = 0.05,
                 FCcutoff = 0.5,
                 subtitle = NULL,
                 x = 'logFC',
                 y = 'adj.P.Val',
                pointSize=1,
                legendLabSize = 10,
                xlim = c(-1.5, 1.5),
                 labSize = 3.0)
```

Example: Volcano Plot (Plot)



Program Goals

- Learn technical skills
- Work with teams to apply skills

Putting It All Together- Project Overview

- *Construction and Analysis of a ceRNA Network Reveals Potential Prognostic Markers in Colorectal Cancer*
  - Using portion of the dataset from the original paper
- Objective: To find differentially expressed genes that act as prognostic markers for colorectal cancer
- Next week: Training using part of the dataset