Module 7
Transcriptomics

Helminth Bioinformatics
Khon Kaen University, 2023

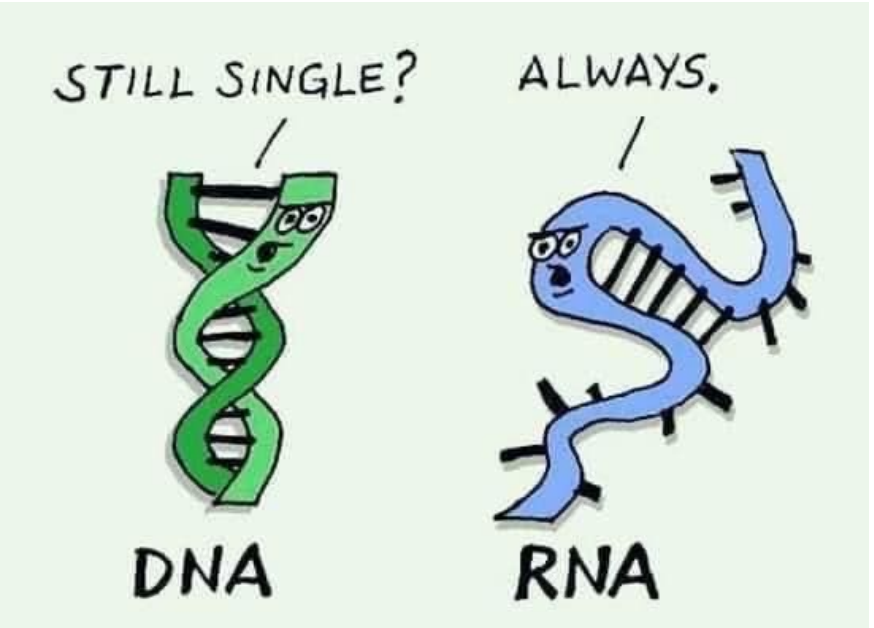
Module aims

You will learn how to:

- map RNA-seq data to reference genome
- acquire read counting results and import them to R
- visualise transcriptomic profiles in R
- using R packages to identify differentially expressed genes and finding patterns in the data
- performing GO term enrichment and interpret the results

What is transcriptome?

All RNA being transcribed
- at a certain developmental stage
- in a certain type of cells
- in response to certain stimuli
- ...



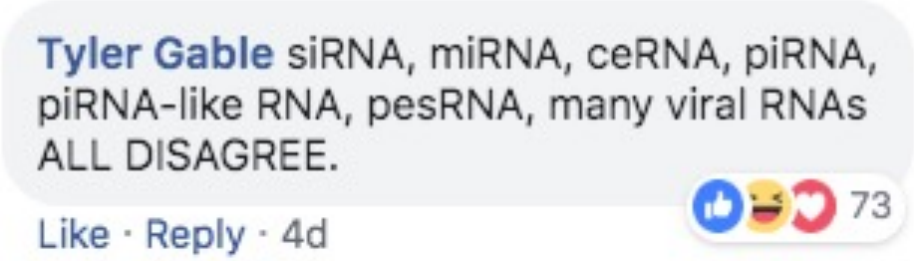
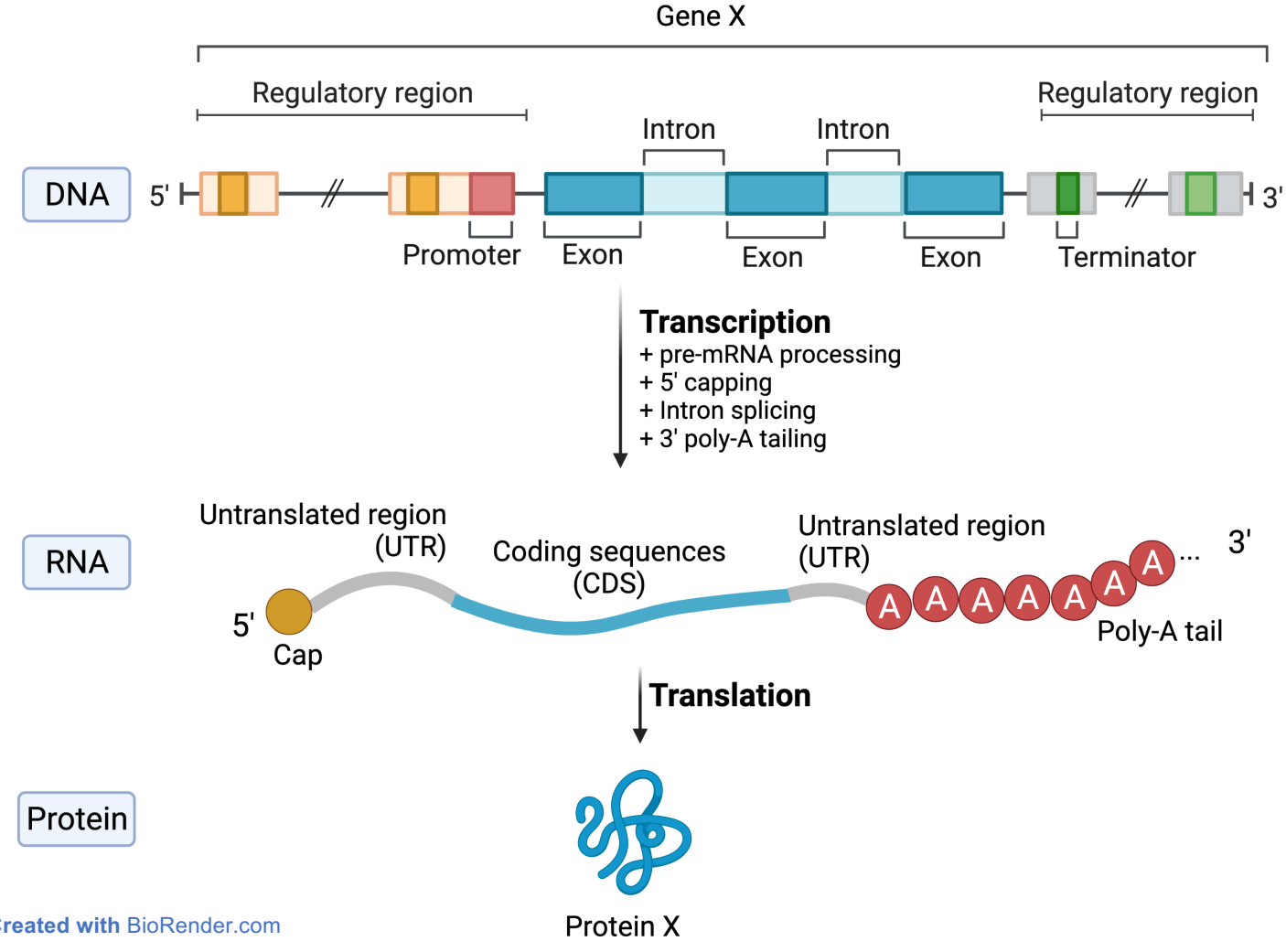


Image from: https://www.facebook.com/trust.biologist/

Eukaryotic gene structure



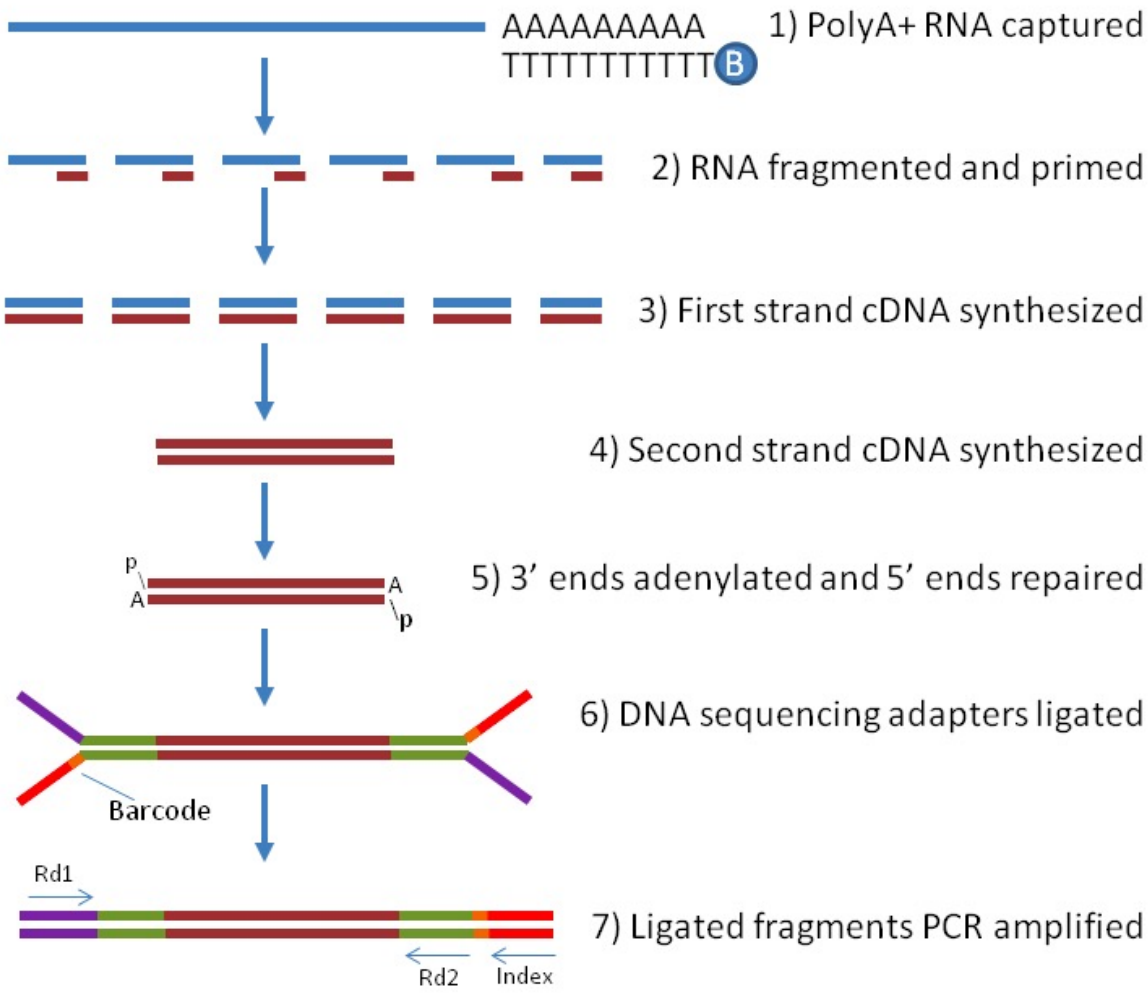


Image from: https://www.labome.com/method/RNA-seq-Using-Next-Generation-Sequencing.html & originally from Corney, D.C. & Basturea, G.N. (2013). Materials and Methods.

What RNA-seq sequences represent

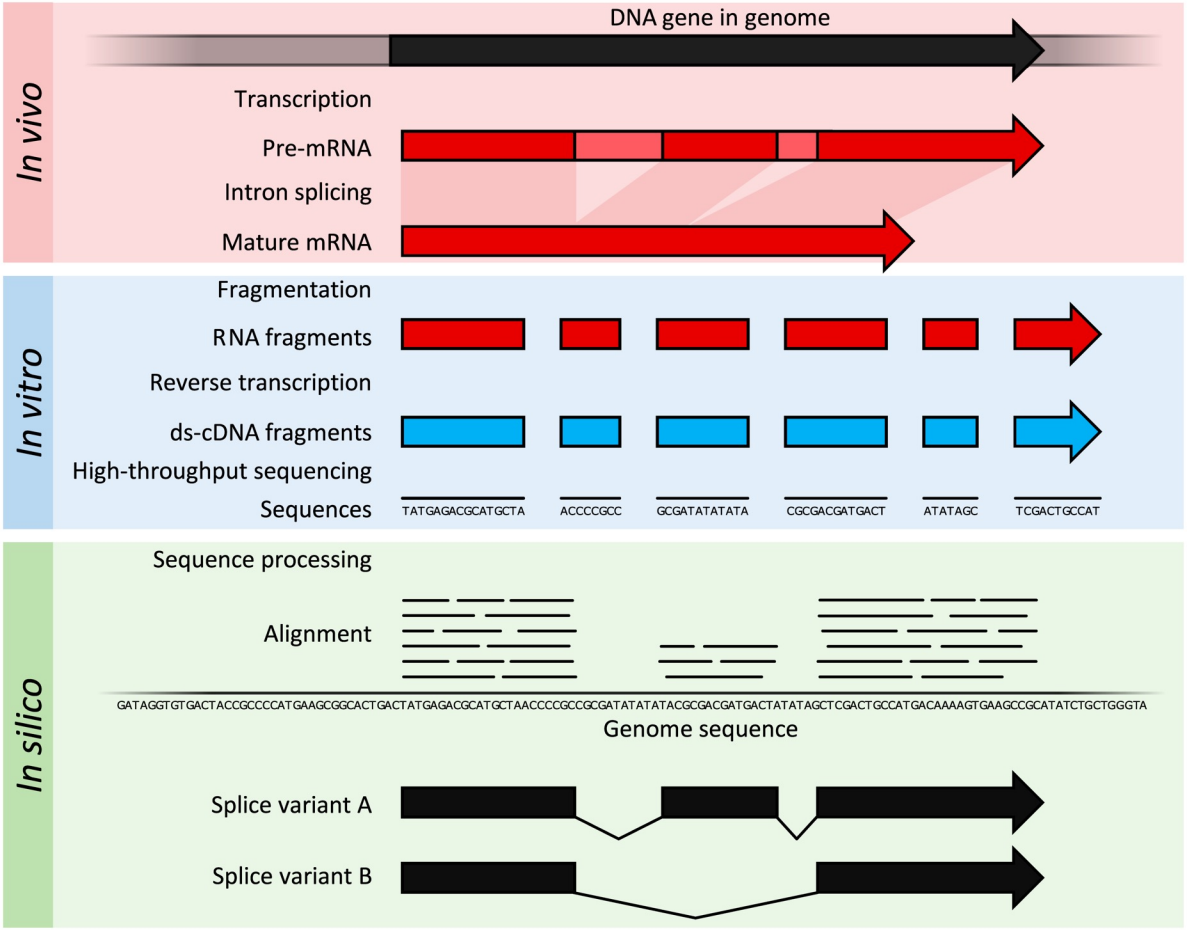


Image from:

Common uses of RNA-seq data

Gene expression study

e.g. differential expression, time course profile

Profiling total RNA (e.g. miRNA and mRNA)

e.g. in exosomes and other secretory products

Splice isoform

only useful for organism with polished reference genomes

SNP calling

use transcriptome as a reduced subset of genomic variation study

Profiling genes in an organism

e.g. for gene annotation, refining gene model

Terms you might come across

number of reads strand-specific single-end/pair-end

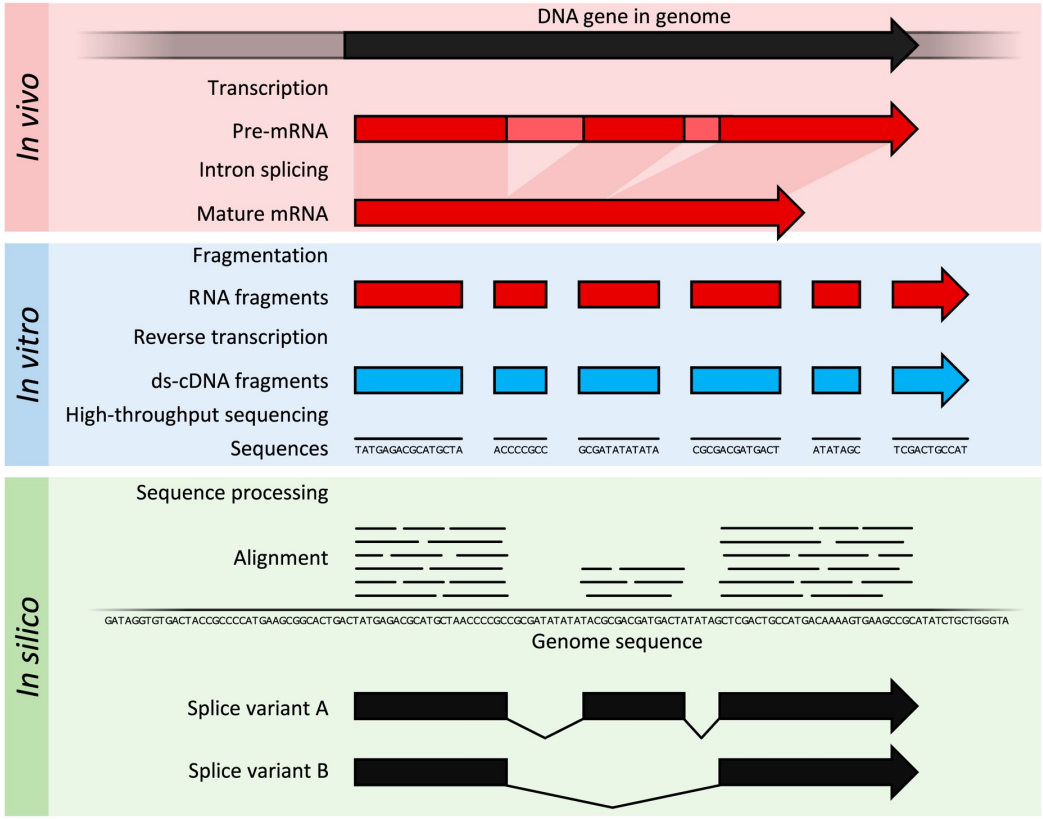
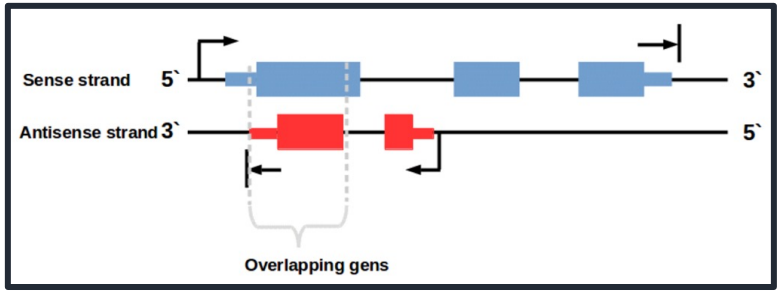


Image from: https://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1005457

Terms you might come across

number of reads **strand-specific** single-end/pair-end

- More reliable quantification of genes on opposite strand
- Allow discovery of anti-sense transcription



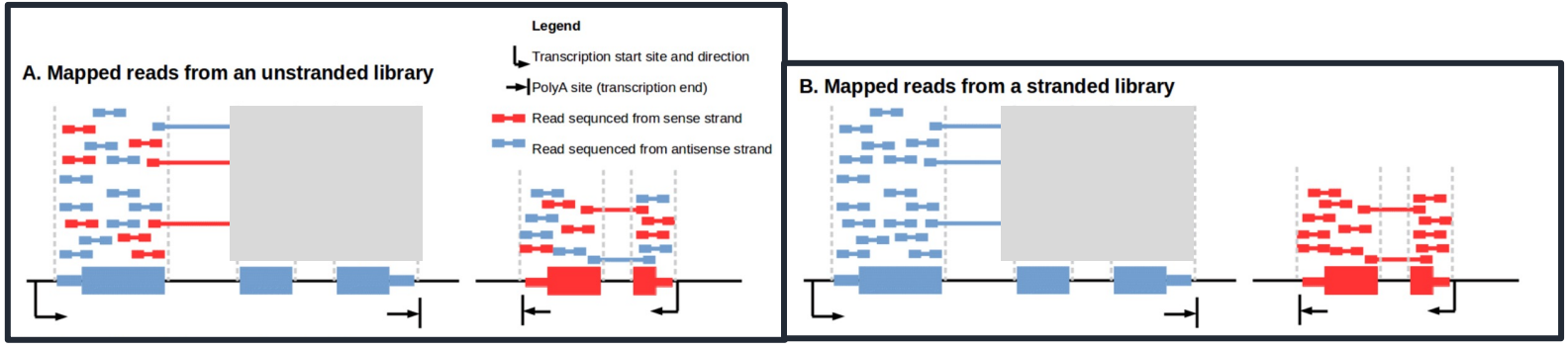


Image from: https://www.ecseq.com/support/ngs/how-do-strand-specific-sequencing-protocols-work

Terms you might come across

number of reads strand-specific **single-end/pair-end**

Single-end

Read fragment from only one end

Can be good enough for gene expression study, if there is a good reference genome

Pair-end

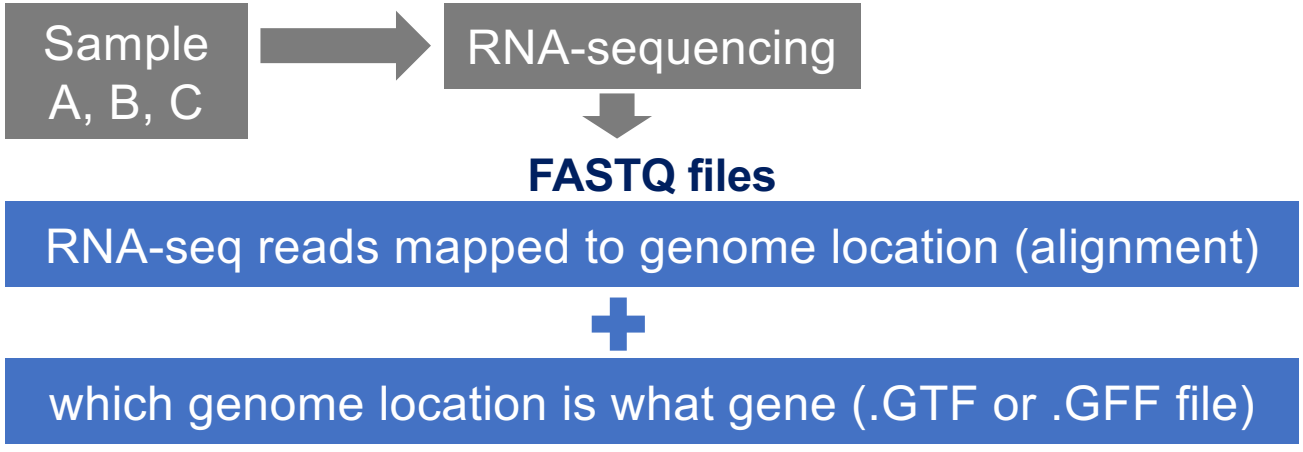
Read from both ends of the fragment

Provide more information which can help with mapping

Highly recommend for organism with only draft reference genome, or wihtout a genome

Image from: https://www.illumina.com/science/technology/next-generation-sequencing/paired-end-vs-single-read-sequencing.html

From sequencing data to read count



Count table

Gene	Count in sample A	Count in sample B	Count in sample C
gene1	4	8	20
gene2	6	3	16
gene3	5	5	15

Almost hands-on time:
genome indexing – why?

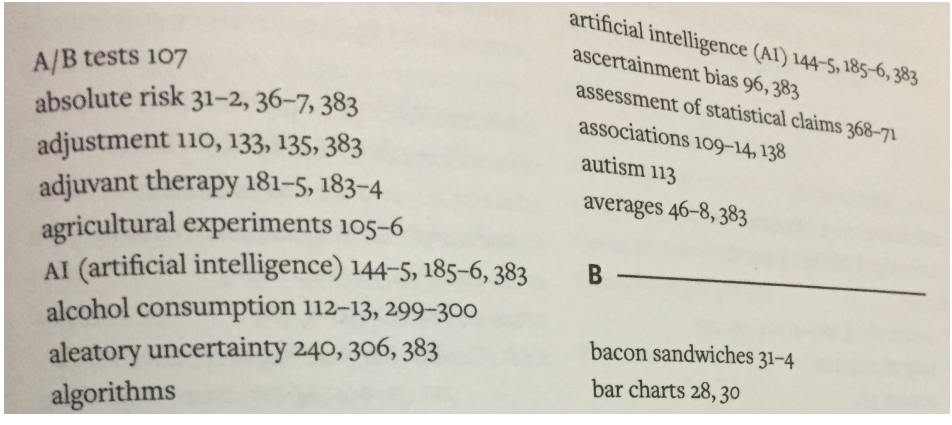
Mapping reads to a genome as approximate pattern matching

Finding your sequences (short texts) in a genome (large book)

Choices

A) Scan the whole genome (large book) for the sequence

B) Pre-process the genome – then searching through book index instead of page by page



Hands-on time!

Index genome using hisat2 (this will take a few minutes)

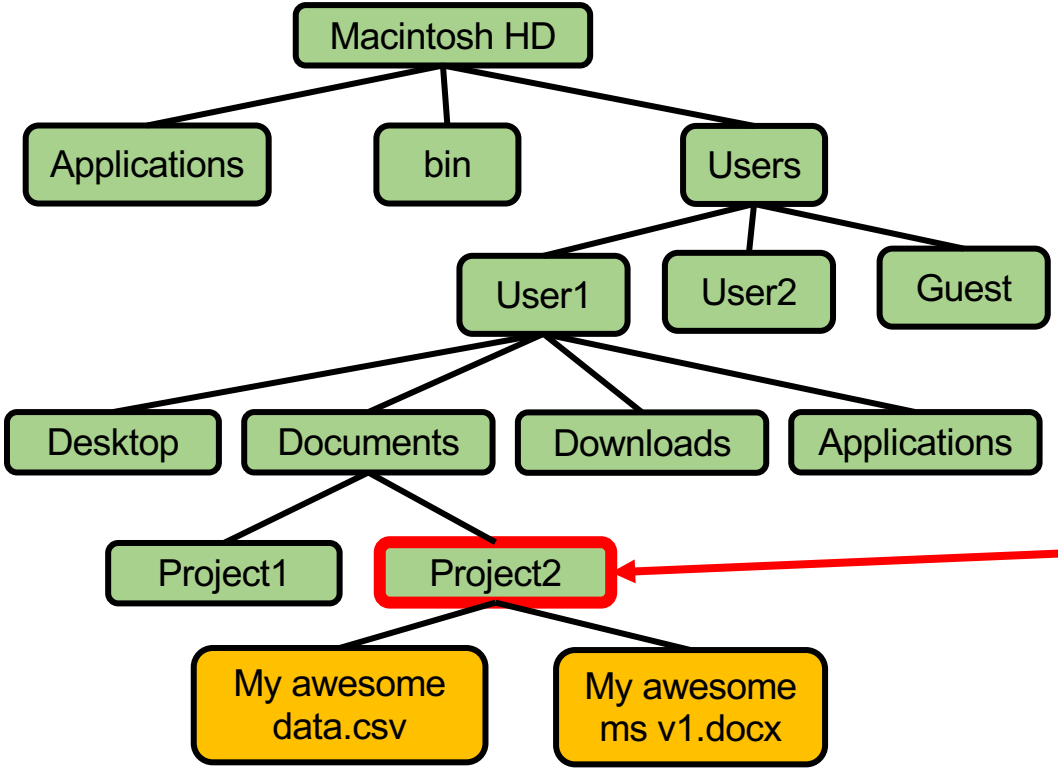
`/location/of/your/data/`

replace text inside with information related to your situation e.g. location of your files

USE TAB (also try double tab)

When copy-paste, check this symbol – and this ”

Mac file system (simplified)



Folder

File

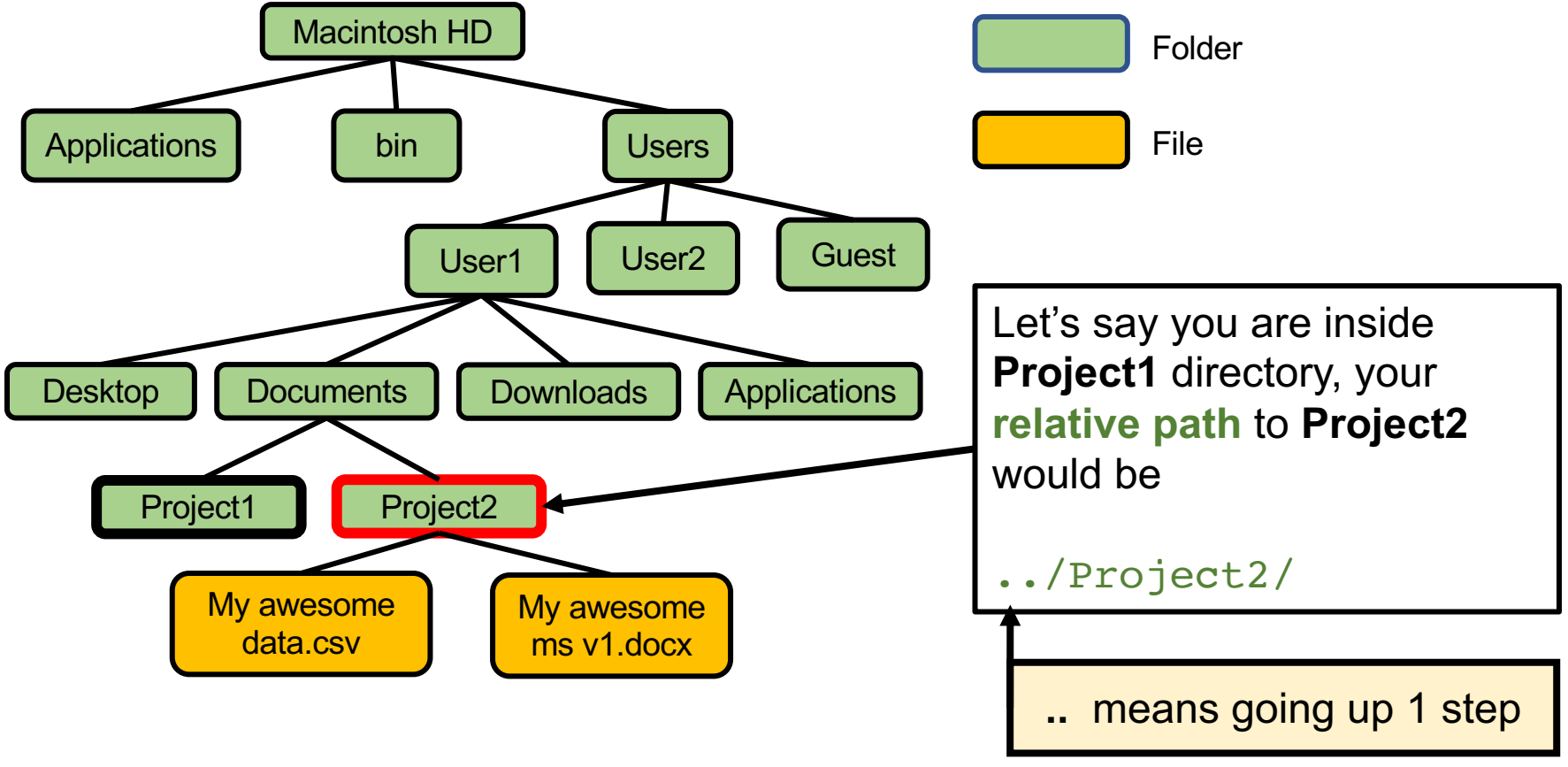
A **full path** to this directory would be

`/Macintosh HD/Users/User1/Documents/Project2/`

or

`~/Documents/Project2/`

Mac file system (simplified)



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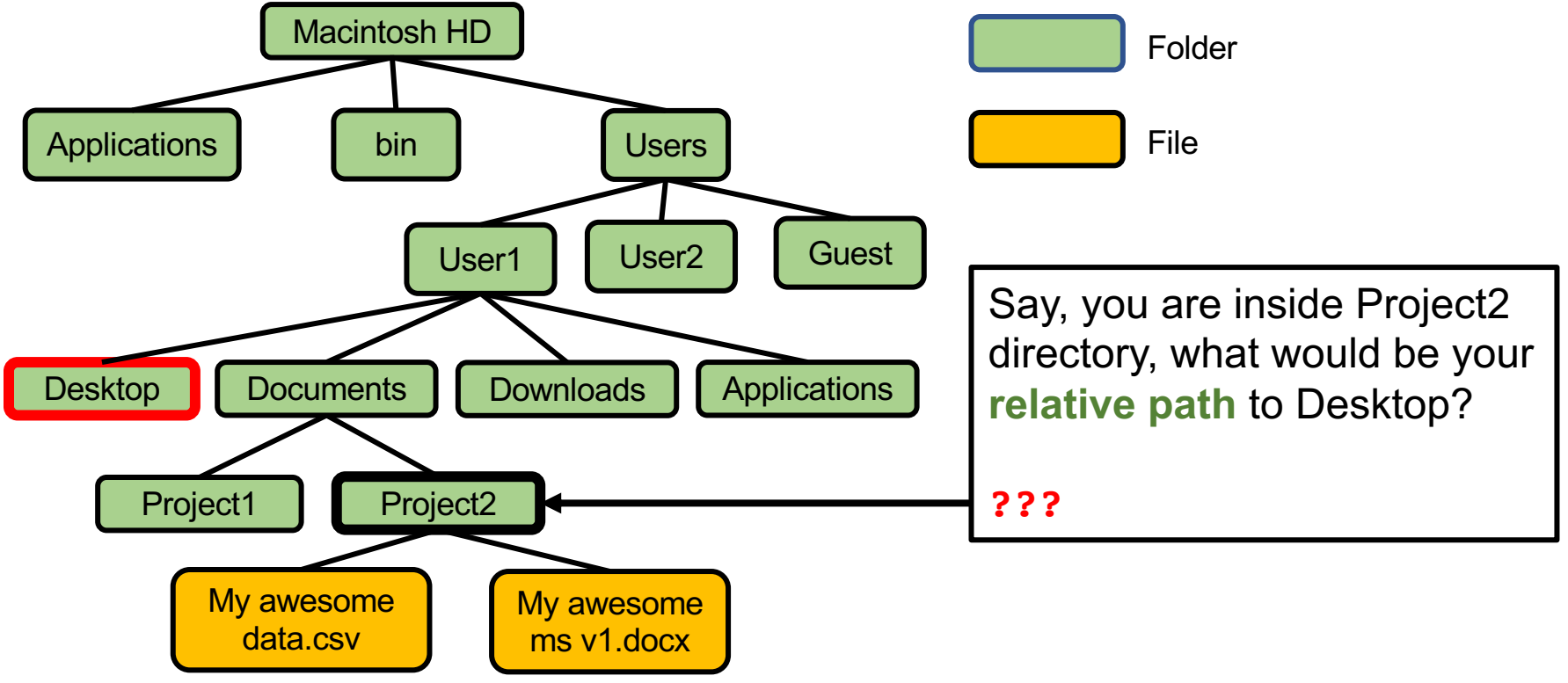

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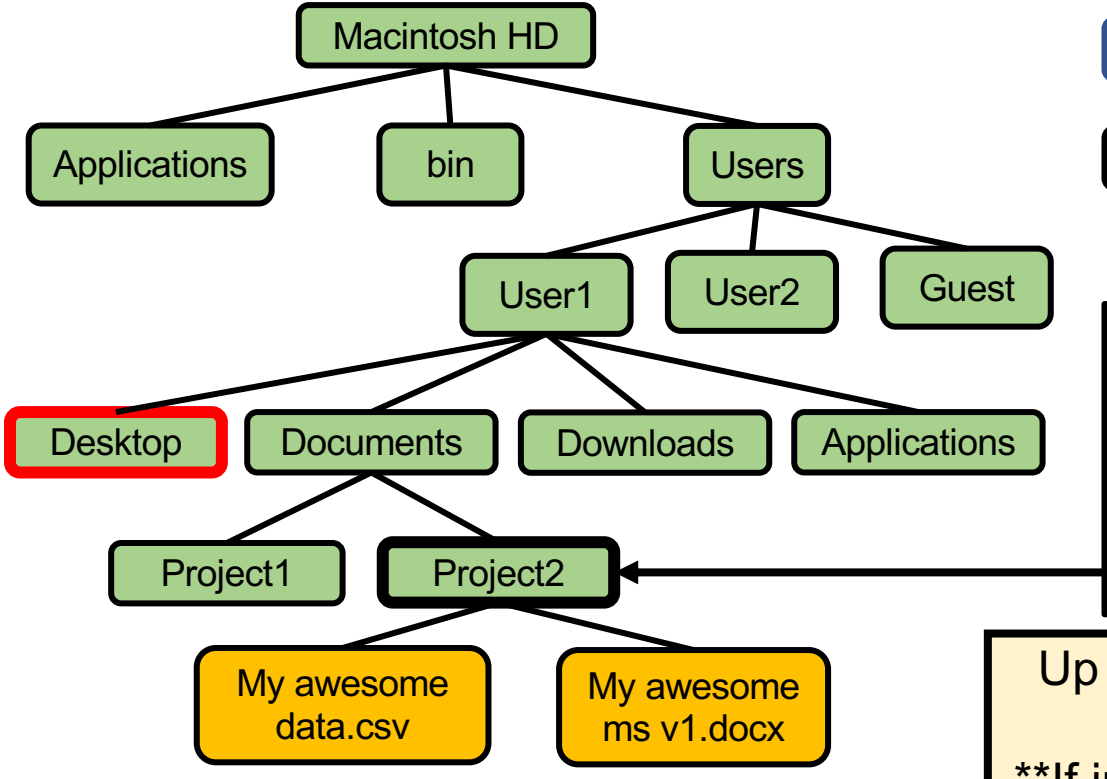


Folder

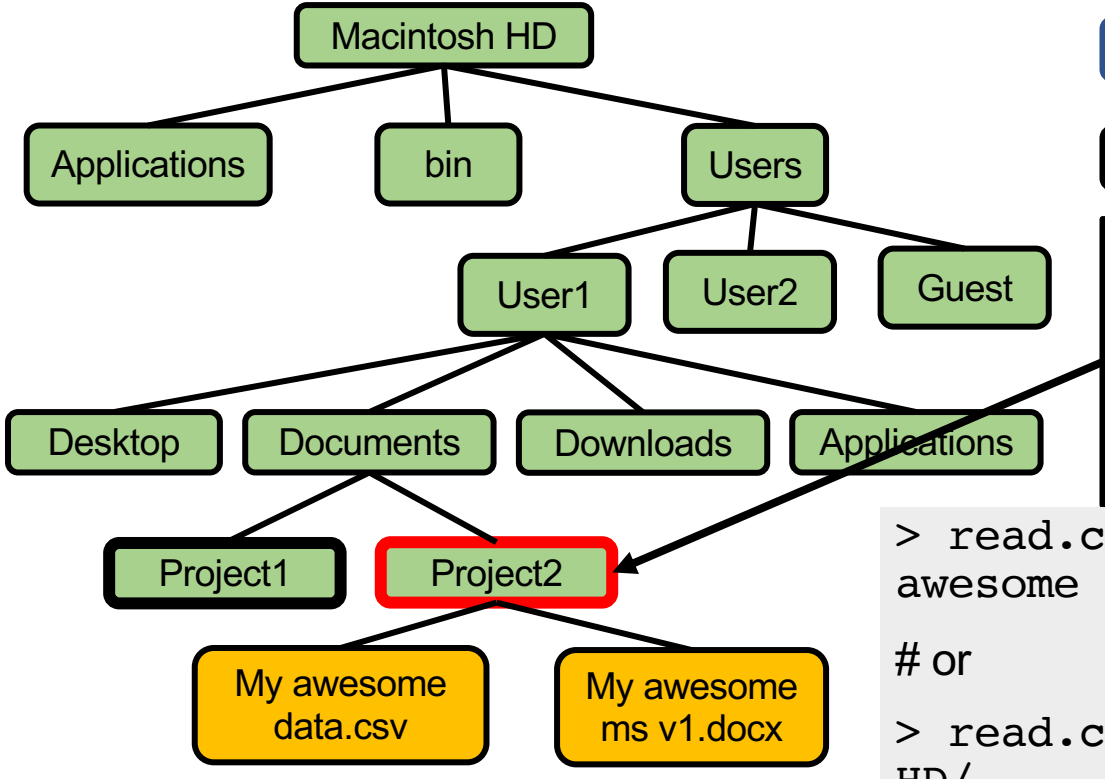
File

Say, you are inside Project2 directory, what would be your **relative path** to Desktop?

`../../Desktop`

Up to **Documents**, up to **User1**, then to **Desktop**
If in doubt, always use full path

Mac file system (simplified)



Folder

File

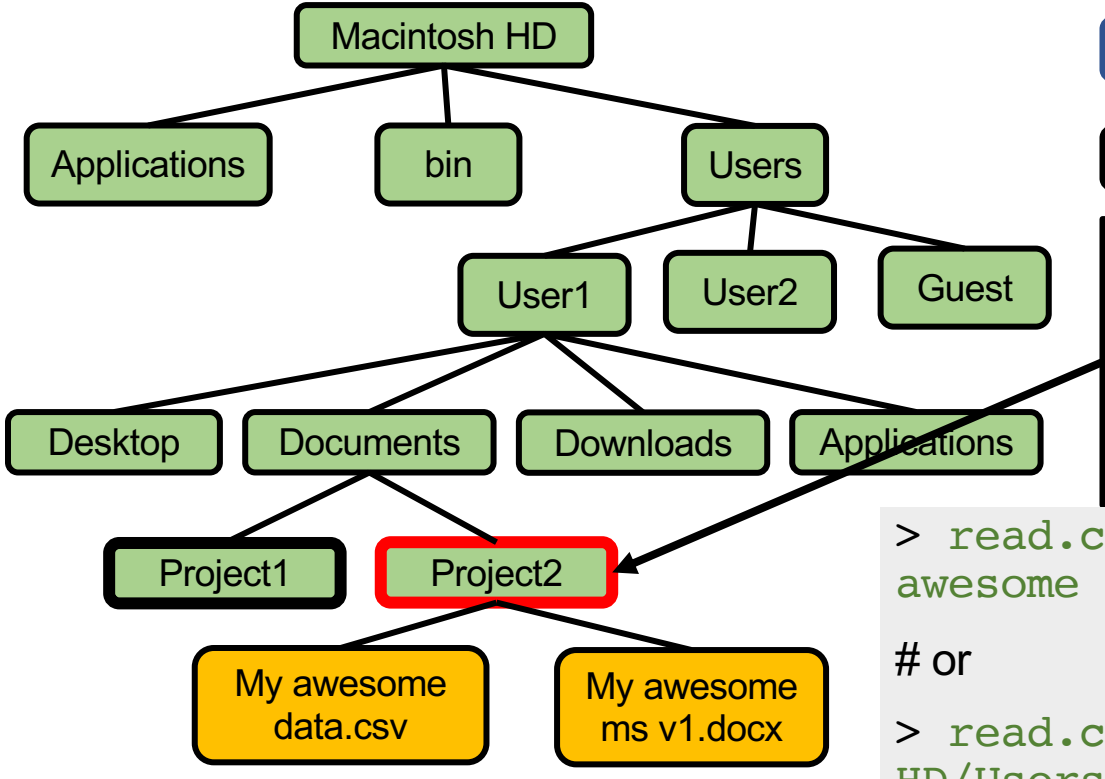
Let's say you are inside **Project1** in R, but you want to import the file "**My awesome data.csv**" which is inside **Project2**

```
> read.csv("../Project2/My awesome data.csv")

# or

> read.csv("/Macintosh HD/_____/My awesome data.csv")
```

Mac file system (simplified)



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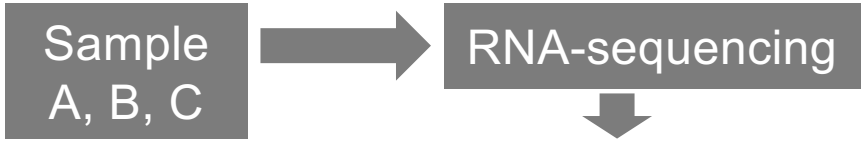
```
> read.csv("../Project2/My awesome data.csv")

# or

> read.csv("/Macintosh HD/Users/User1/Documents/Project 2/My awesome data.csv")
```

What we did in unix

- Genome indexing
- Map (align) reads to genome
  - SAM & BAM files
- Get read counts per gene
  - (*_v10.count)

From sequencing data to read count



FASTQ files

RNA-seq reads mapped to genome location (alignment)

+

which genome location is what gene (.GTF or .GFF file)

Count table

Gene	Count in sample A	Count in sample B	Count in sample C
gene1	4	8	20
gene2	6	3	16
gene3	5	5	15

```
$ head *.count
==> D06_1_v10.count <==
Smp_000020.1 299
Smp_000030.1 1071
Smp_000040.1 425
Smp_000050.1 190
Smp_000070.1 156

==> D06_2_v10.count <==
Smp_000020.1 76
Smp_000030.1 310
Smp_000040.1 134
Smp_000050.1 67
Smp_000070.1 46
```

Next.. R

- Prepare data for analysis in R
- Identify differentially expressed (DE) genes
- Create plots
- Functional analysis

Fold change

A (D13)

B (D06)

Log_2FC (log_2 of fold change)

$$log_2\left(\frac{A(D13)}{B(D06)}\right)$$

Log_2FC (log_2 of fold change)

$$log_2\left(\frac{8}{2}\right)$$

Log_2FC (log_2 of fold change)

$$log_2\left(\frac{A(D13)}{B(D06)}\right)$$

Log_2FC (log_2 of fold change)

$$log_2\left(\frac{2}{8}\right)$$

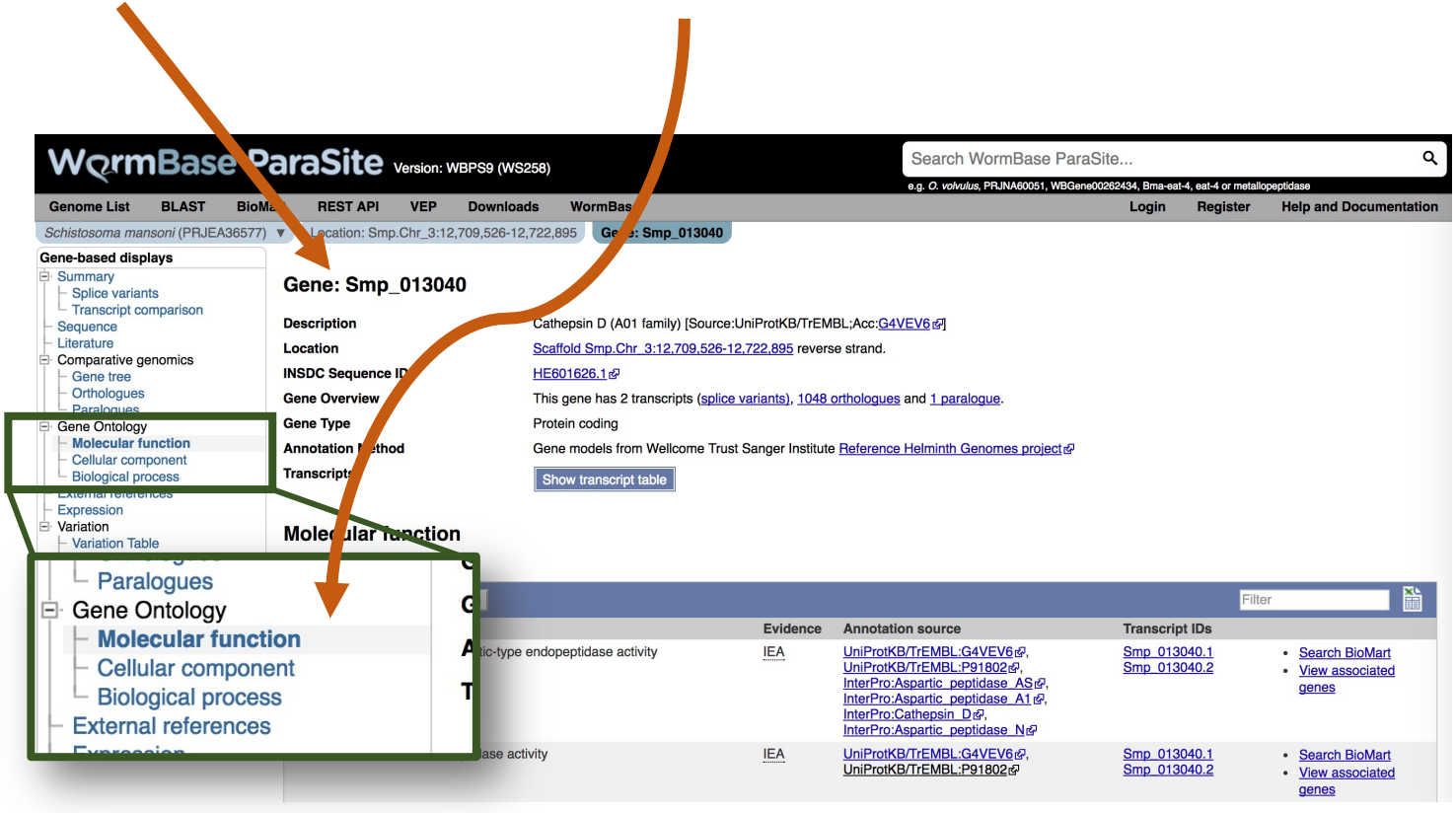
Functional analysis

- Rather than going through the list of differentially expressed genes to find genes that you expect to see changes
  - Do functional analysis
  - Let data guide the way

- Possibly the most common = GO enrichment

GO term enrichment

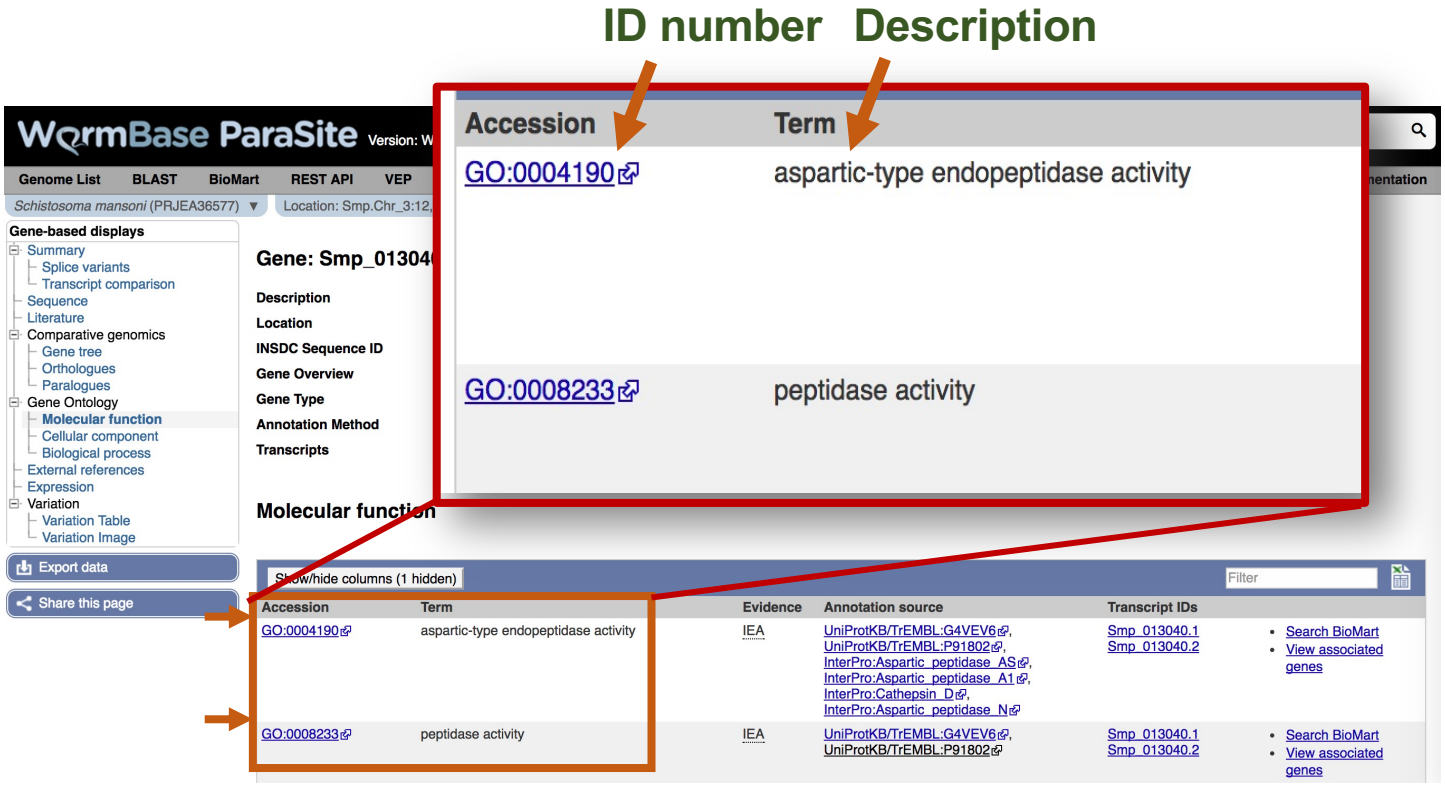
Genes often have associated GO terms (Gene Ontology terms).



GO term enrichment

Genes often have associated GO terms (Gene Ontology terms).

GO terms describe functions of a gene, and can be derived from sequence similarity, experiment, homology etc.



GO term enrichment

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GO term enrichment: “Are there any GO terms present in my data more frequently than expected by chance alone?”