Introduction II: tools you need to analyse sequencing dataset

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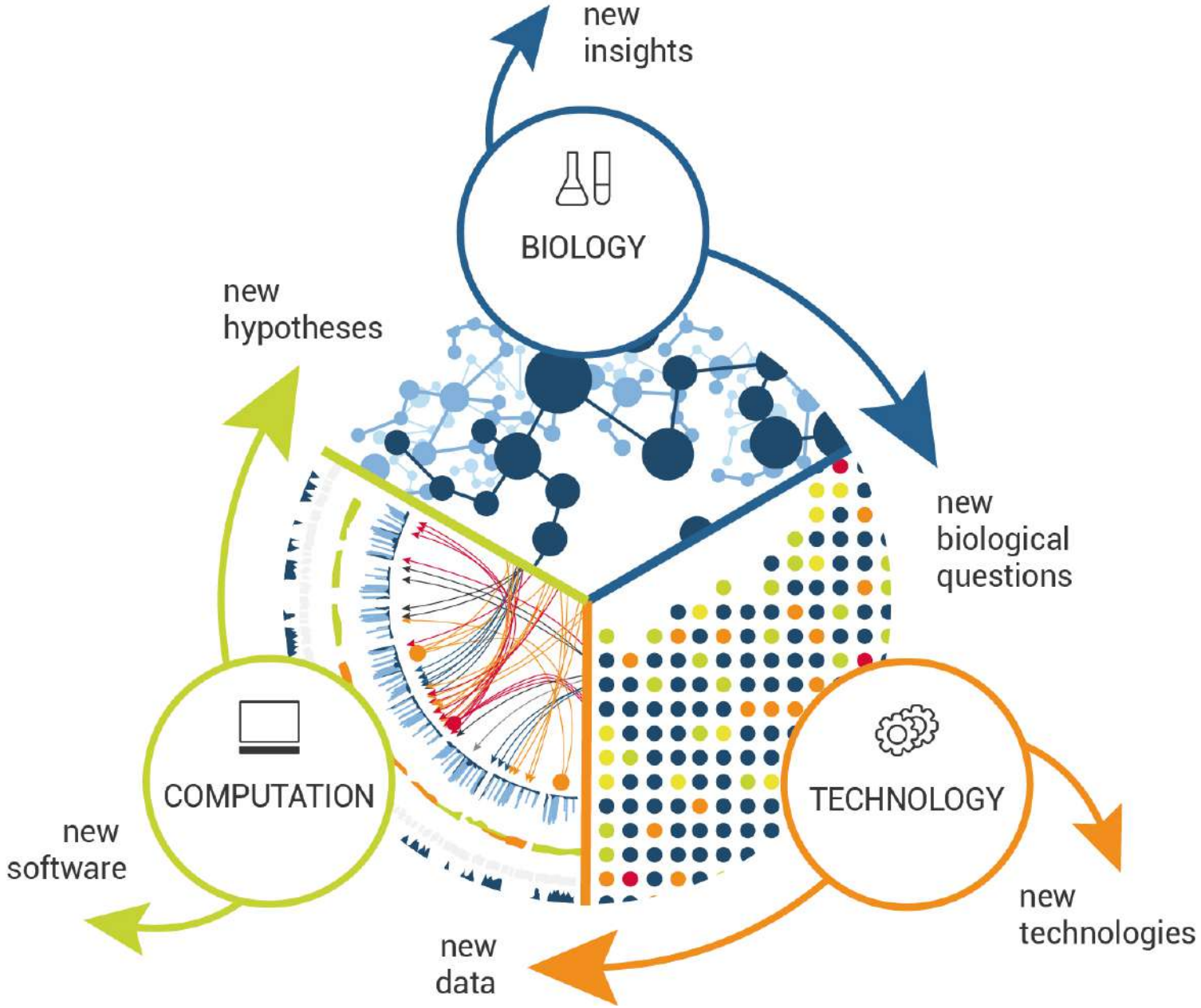
Introduction to NGS Data and Analysis
Lecture 2 ; v2020

This lecture aims to expose you to how computational biologists' way of thinking (and any small topics that relevant)

The content of this lecture will not be in the final exam. There is a written homework assignment.

Lecture outline

1. Research Effectively
2. Linux
3. Keep tracking / Reproducible research
4. Data Type / Visualisations
5. R



Credit: http://www.omicscouts.com

COMMENTARY

_computational BIOLOGY

So you want to be a computational biologist?

Nick Loman & Mick Watson

Two computational biologists give advice when starting out on computational projects.

https://www.nature.com/articles/nbt.2740.pdf

Table 1 Essential tools for the biological software developer

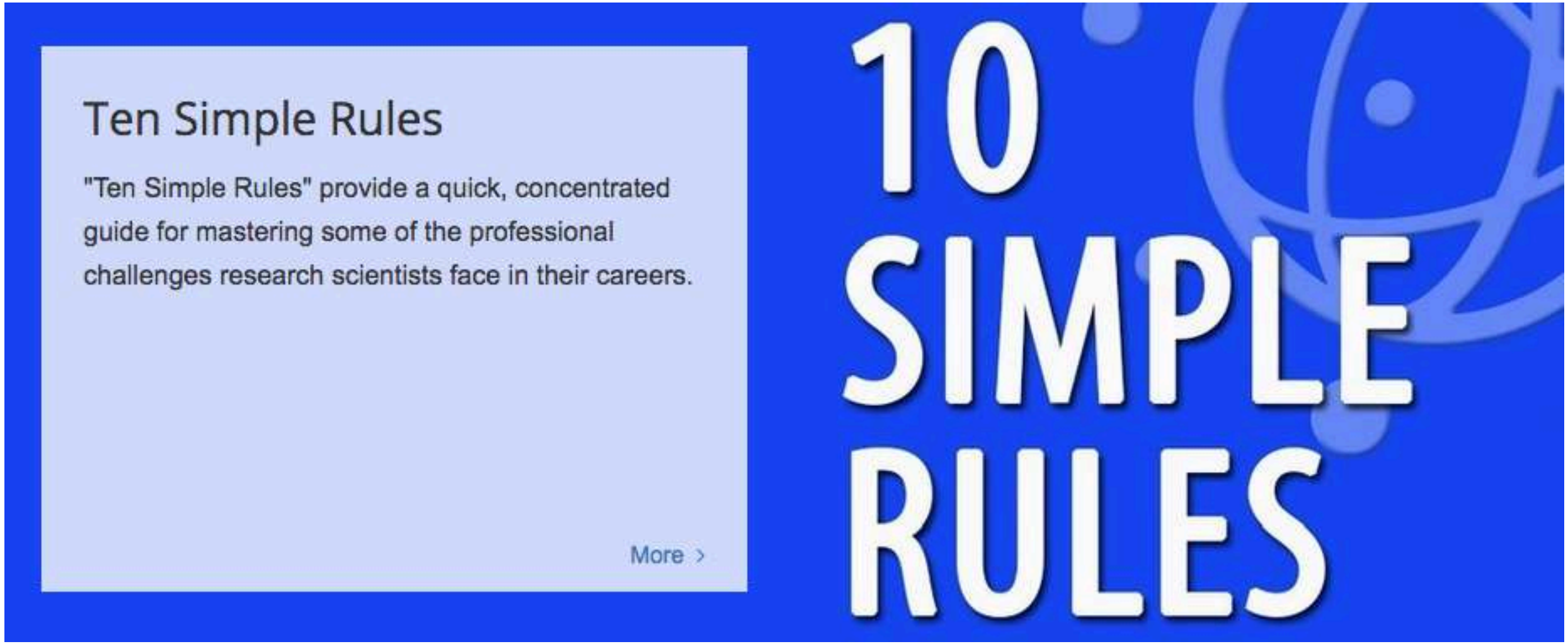
Task	Tools
Collaborative software development	Share data and code through online collaborative working environments such as Github, Sourceforge and Bitbucket. Use Google to find tutorials on these systems, e.g., http://try.github.io/
Build powerful pipelines	There are modern software libraries, such as Ruffus, and more traditional tools, such as Make, to build pipelines from existing software tools. Your choice will depend on personal preference and on your favorite programming language.
Make your pipelines available	You may be comfortable on the command line, but your collaborators may not be. Therefore you can deliver your pipelines through graphical environments such as Galaxy (http://www.galaxyproject.org/) or Taverna (http://www.taverna.org.uk/).
Integrated development environment (IDE)	Whether you want to adopt a full IDE, such as Eclipse, or an advanced text editor, such as Emacs, you will need something to use to develop your code. Again, this will likely depend on your choice of language and personal preference. However, at some point, you'll have to use a command line–based editor, such as vim or nano, so it's advisable to learn at least the basics.

https://www.nature.com/articles/nbt.2740.pdf

Table 2 Useful resources for learning

Type of information	Relevant URLs
MOOCs (massive open online courses)	These are very popular at the moment and offer free training over the internet. Coursera (https://www.coursera.org/), Udacity (https://www.udacity.com/), edX (https://www.edx.org/) and the Kahn Academy (https://www.khanacademy.org/) have a range of courses relevant to bioinformatics, genomics, computing, statistics and modeling.
Learning to code	Codecademy (http://www.codecademy.com/) and Code School (https://www.codeschool.com/) are not specific to biology but do offer simple ways to learn how to code. For a more biological perspective, "Python for biologists" (http://pythonforbiologists.com/) is always popular. For examples of best practices visit http://software-carpentry.org/.
Bioinformatics problem solving	Learn bioinformatics through problem solving and pit your wits against others at http://www.rosalind.info.
Web forums	These are essential when you start out—ask questions and receive answers from experts at http://www.seqanswers.com/ and http://www.biostars.org/.
International organizations	GOBLET is the global organization for bioinformatics learning education and training (http://www.mygoblet.org/), and ELIXIR is a European organization set up to provide an infrastructure, including training, for life sciences information (http://www.elixir-europe.org/).
Blogs and lists	A variety of blogs and lists exist online that detail computational biology courses, such as http://stephenturner.us/p/edu and http://ged.msu.edu/angus/bioinformatics-courses.html.

https://www.nature.com/articles/nbt.2740.pdf



http://collections.plos.org/ten-simple-rules

Research effectively / Data management

Thomas D. Otto

University of Glasgow
Verified email at glasgow.ac.uk - Homepage
Big Data Algorithms Omics

17 papers in 2017 ; how?
(I know he's doing the work)

In silico guided reconstruction and analysis of ICAM-1-binding var genes from Plasmodium falciparum E Carrington, TD Otto, T Szestak, F Lennartz, MK Higgins, CI Newbold, ... Scientific reports 8 (1), 3282		2018
Genomes of all known members of a Plasmodium subgenus reveal paths to virulent human malaria TD Otto, A Gilabert, T Crellen, U Böhme, C Arnathau, M Sanders, S Oyola, ... bioRxiv, 095679	2	2018
Complete avian malaria parasite genomes reveal features associated with lineage specific evolution in birds and mammals U Boehme, TD Otto, J Cotton, S Steinbiss, M Sanders, SO Oyola, A Nicot, ... BioRXiv, 086504	5	2018
A hybrid-hierarchical genome assembly strategy to sequence the invasive golden mussel Limnoperna fortunei M Uliano-Silva, F Dondero, T Dan Otto, I Costa, NCB Lima, JA Americo, ... GigaScience	1	2017
Profiling invasive Plasmodium falciparum merozoites using an integrated omics approach K Kumar, P Srinivasan, MJ Nold, JK Moch, K Reiter, D Sturdevant, TD Otto, ... Scientific reports 7 (1), 17146		2017
PIGGYBAC MUTAGENESIS SCREENING OF THOUSANDS OF PLASMODIUM FALCIPARUM GENES REVEALS WHAT A MALARIA PARASITE CAN'T LIVE WITHO... M Zhang, C Wang, TD Otto, J Oberstaller, IF Bronner, S Li, K Udenze, ... AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE 95 (5), 390-391		2017
WHOLE GENOME SEQUENCING OF PLASMODIUM FALCIPARUM MALARIA PARASITES FROM DRIED BLOOD SPOTS: GATEWAY TO HIGH-RESOLUTION GEN... CV Ariani, WL Hamilton, S Oyola, LN Amenga-Etego, M Kekre, ... AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE 95 (5), 391-391		2017
Genomic characterization of recrudescent Plasmodium malariae after treatment with artemether/lumefantrine GG Rutledge, I Marr, GKL Huang, S Auburn, J Marfurt, M Sanders, ... Emerging infectious diseases 23 (8), 1300	1	2017
Human vaccination against Plasmodium vivax Duffy-binding protein induces strain-transcending antibodies RO Payne, SE Silk, SC Elias, KH Milne, TA Rawlinson, D Llewellyn, ... JCI insight 2 (12)	3	2017
A single nucleotide polymorphism in an AP2 transcription factor encoded in the malaria-causing Plasmodium berghei alters the development of host immunity PW Sheehan, M Akkaya, A Bansal, G Arora, TD Otto, CF Qi, M Pena, ... The Journal of Immunology 198 (1 Supplement), 77.5-77.5		2017
pfk13-independent treatment failure in four imported cases of Plasmodium falciparum malaria treated with artemether-lumefantrine in the United Kingdom CJ Sutherland, P Lansdell, M Sanders, J Muwanguzi, DA van Schalkwyk, ... Antimicrobial agents and chemotherapy 61 (3), e02382-16	11	2017
Plasmodium malariae and P. ovale genomes provide insights into malaria parasite evolution GG Rutledge, U Böhme, M Sanders, AJ Reid, JA Cotton, ... Nature 542 (7639), 101	22	2017
SC83288 is a clinical development candidate for the treatment of severe malaria S Pegoraro, M Duffey, TD Otto, Y Wang, R Rösemann, R Baumgartner, ... Nature communications 8, 14193	4	2017
Correction: Variant Exported Blood-Stage Proteins Encoded by Plasmodium Multigene Families Are Expressed in Liver Stages Where They Are Exported into the Pa... A Fougère, AP Jackson, DP Bechtsi, JAM Braks, T Annoura, J Fonager, ... PLoS pathogens 13 (1), e1006128		2017
A SATURATION-LEVEL PIGGYBAC MUTAGENESIS SCREEN OF THE PLASMODIUM FALCIPARUM GENOME DEFINES GENES IMPORTANT FOR IN VITRO ASE... M Zhang, C Wang, J Oberstaller, TD Otto, S Adapa, X Liao, J Swanson, ... AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE 97 (5), 19-20		2017
A LARGE-SCALE GENETIC SCREEN OF PLASMODIUM FALCIPARUM IDENTIFIES GENOTYPY-PHENOTYPE MUTATIONS AFFECTING TOLERANCE TO FEBRIL... M Zhang, C Wang, P Thomas, J Oberstaller, TD Otto, X Liao, S Li, ... AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE 97 (5), 323-323		2017
ESSENTIAL ASPECTS OF RNA METABOLISM FOR P. FALCIPARUM BLOOD-STAGE SURVIVAL J Oberstaller, M Zhang, CQ Wang, TD Otto, X Liao, J Swanson, SR Adapa, ... AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE 97 (5), 625-625		2017
Integrated pathogen load and dual transcriptome analysis of systemic host-pathogen interactions in severe malaria HJ Lee, M Walther, A Georgiadou, D Nwakanma, LB Stewart, M Levin, ... bioRxiv, 193631		2017
Plasmodium vivax-like genome sequences shed new insights into Plasmodium vivax biology and evolution A Gilabert, T Otto, G Rutledge, B Franzon, B Ollomo, C Arnathau, ... bioRxiv, 205302		2017
An improved Plasmodium cynomolgi genome assembly reveals an unexpected methyltransferase gene expansion EM Pasini, U Böhme, GG Rutledge, A Voorberg-Van der Wel, M Sanders, ... Wellcome open research 2	3	2017


Don't splash water on my laptop
Now, you're exaggerating
BioinFormaticians: They can work From anywhere
www.biocomicals.com


How are the analysis coming?
Almost ready
www.biocomicals.com

What do we actually do everyday?

- You have got new data!
  - **(1) Need to understand, QC, and analyse the data**. How?

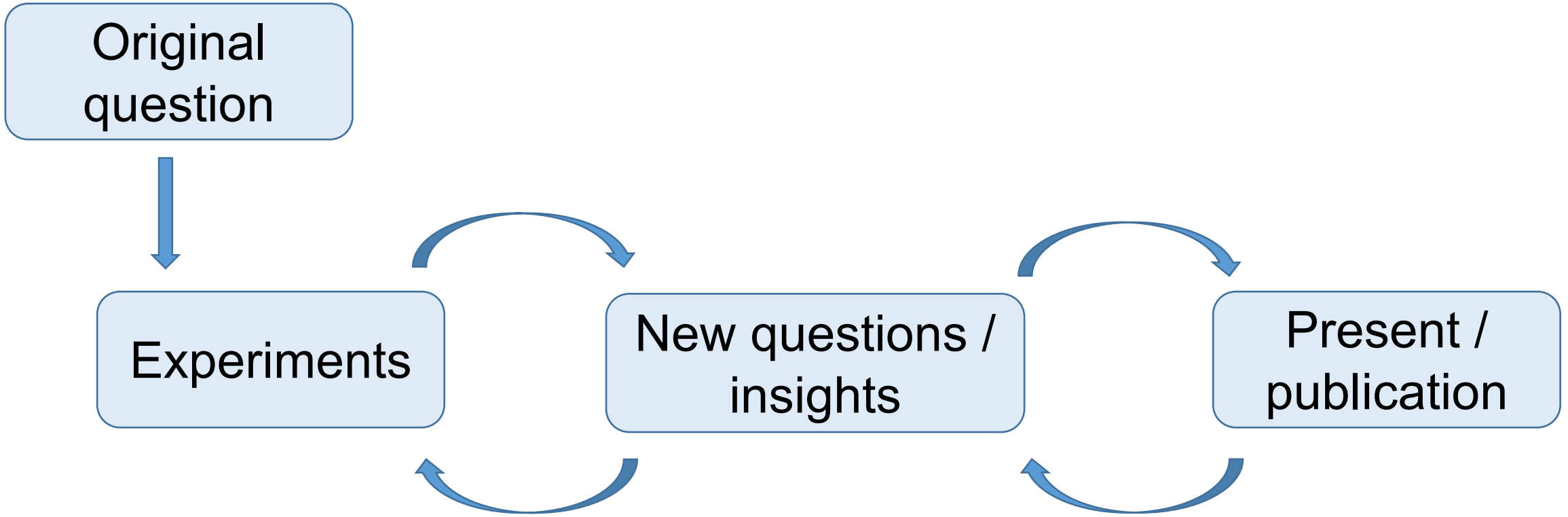
- Once the data has been **explored**, you need to compare against published ones
  - **(2)** You need to survey, and download the right dataset
  - Move to step **(1)**

- **(3)** Then you need to **visualise**
- Does it answer your question? There are times when you need to **(4)** develop new/better algorithms and **(5)** generate more data

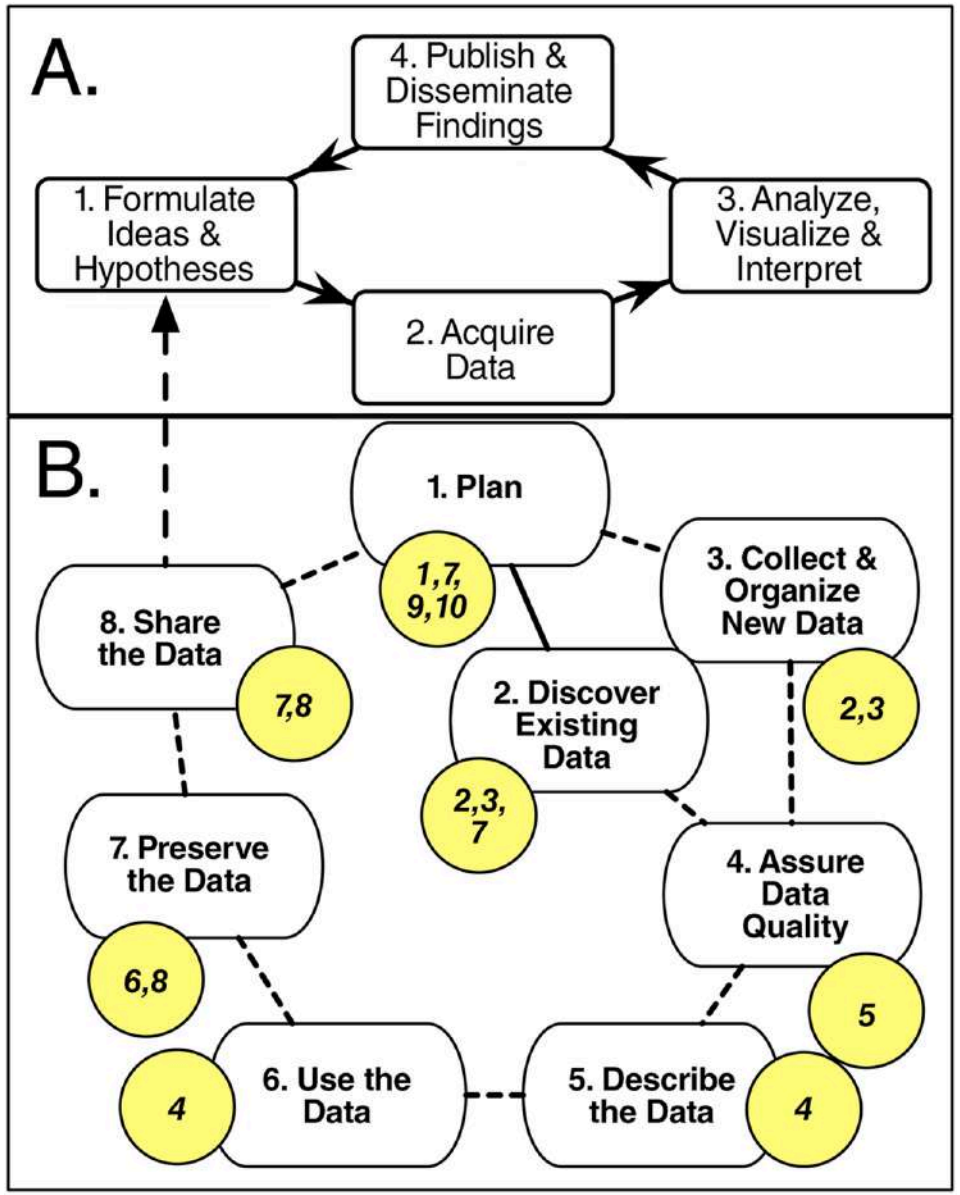
Finally to present to an audience

- Remember to save all your work first!
  - Organisation and record-keeping
- Publications? But before that…
- Are the data shared to the public?
  - How?
- Are the results reproducible?
  - How?

Experimental Analysis

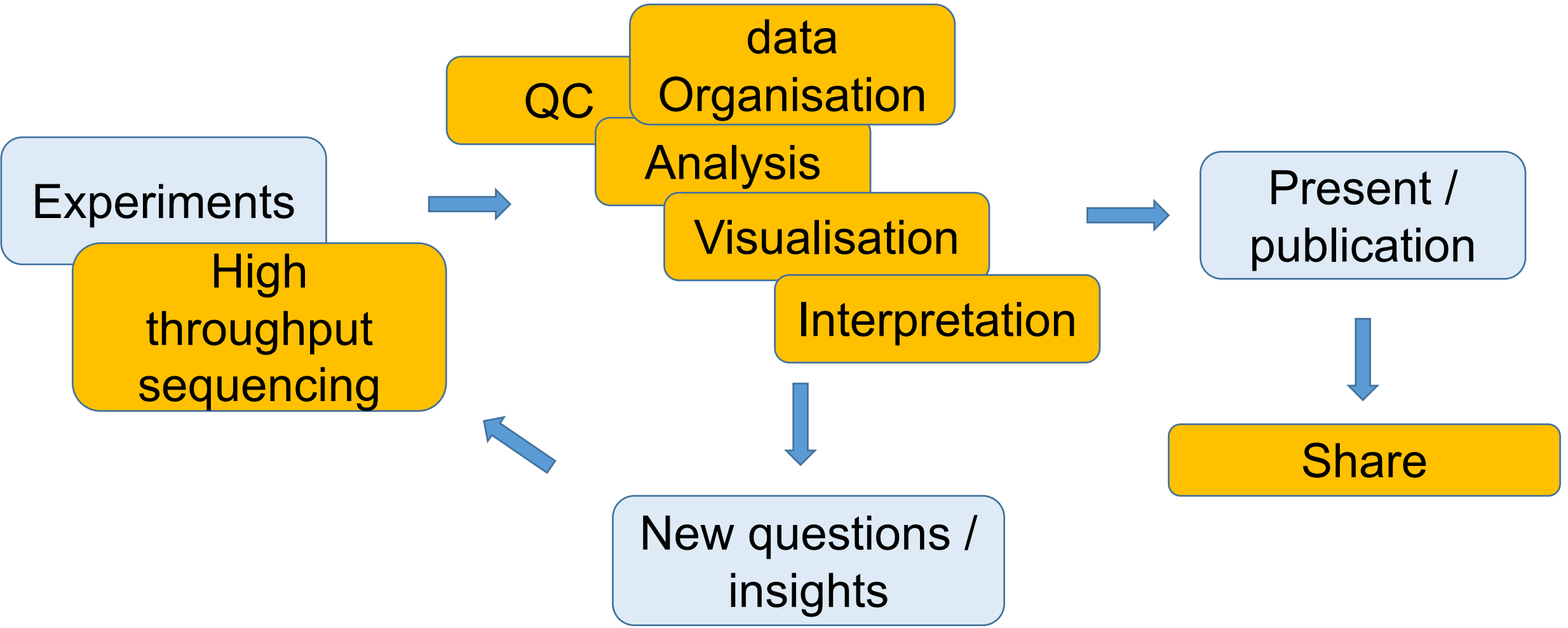


Relationship of the research life cycle (A) to the data life cycle (B)



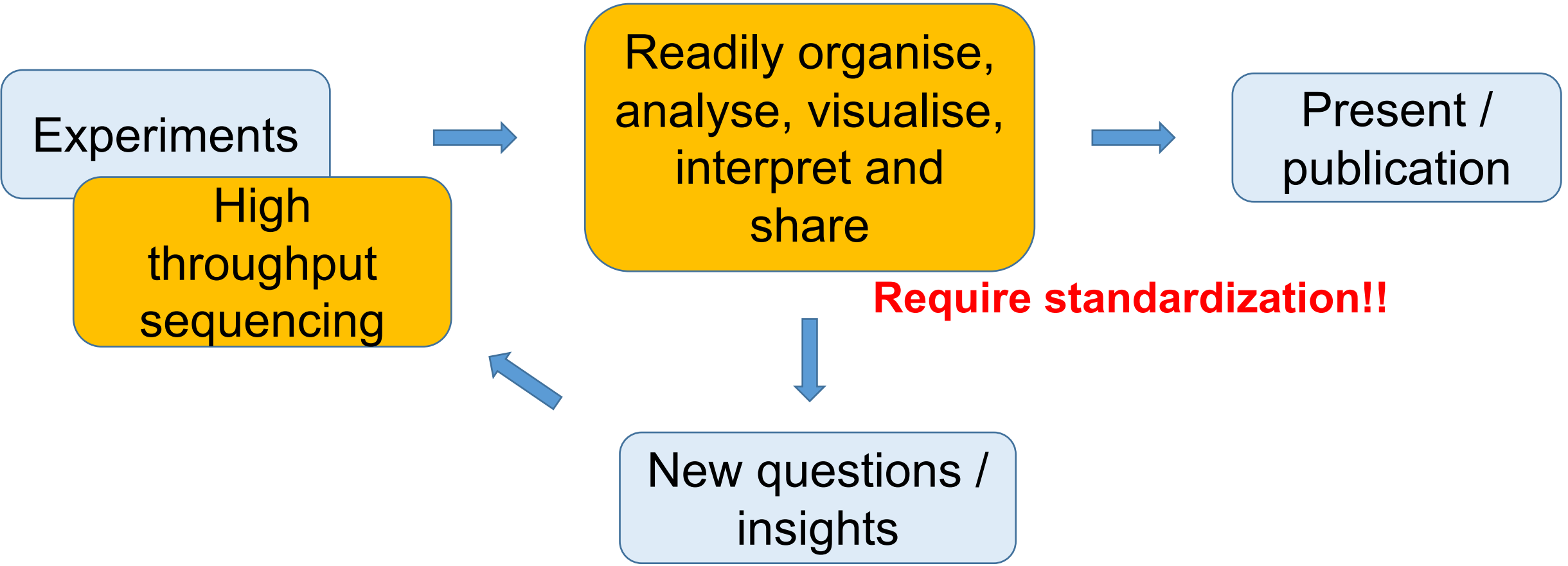
- https://doi.org/10.1371/journal.pcbi.1004525

Analysis in a high throughput world: challenges



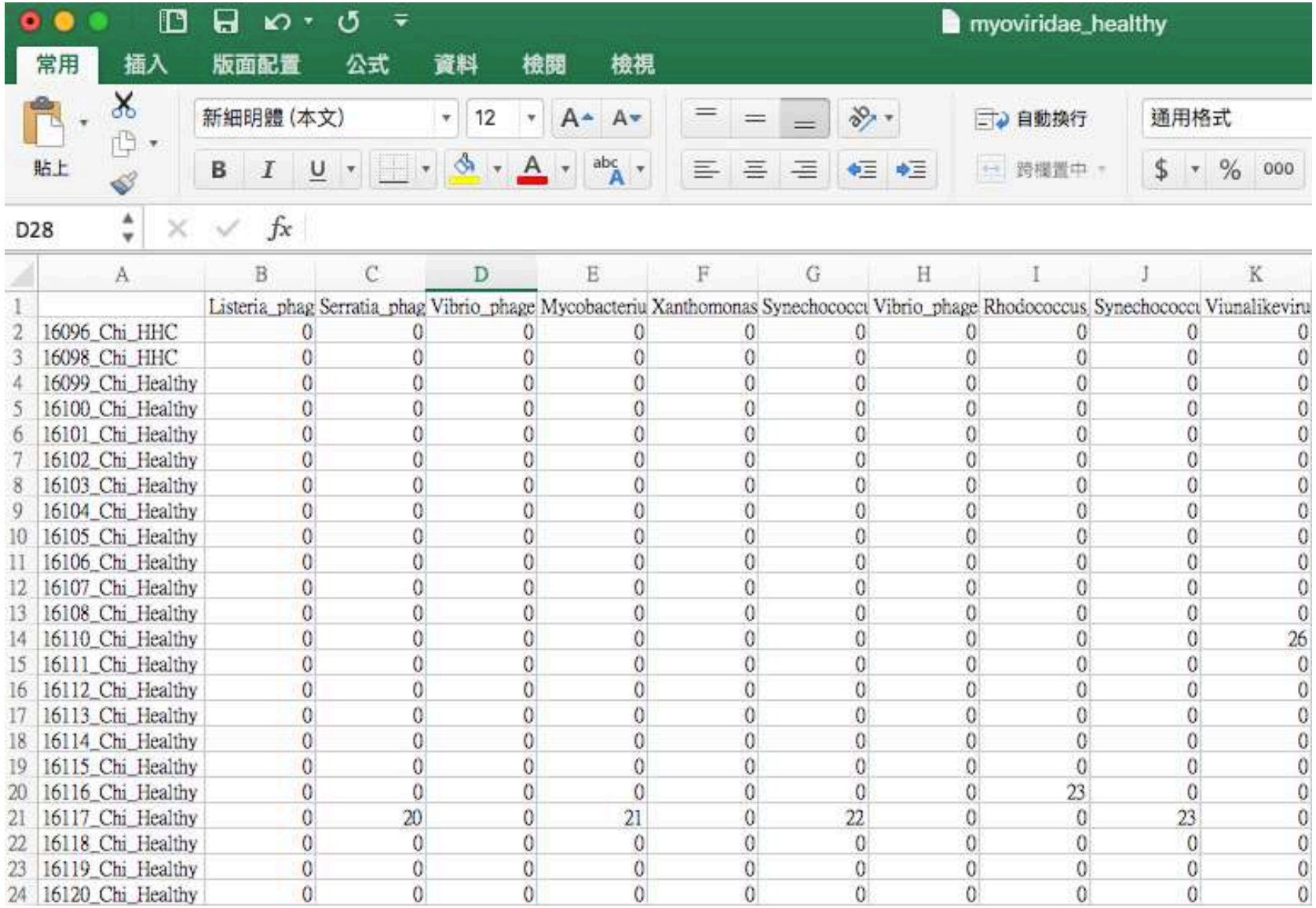
x10-30

Analysis in a high throughput world: reorganisation

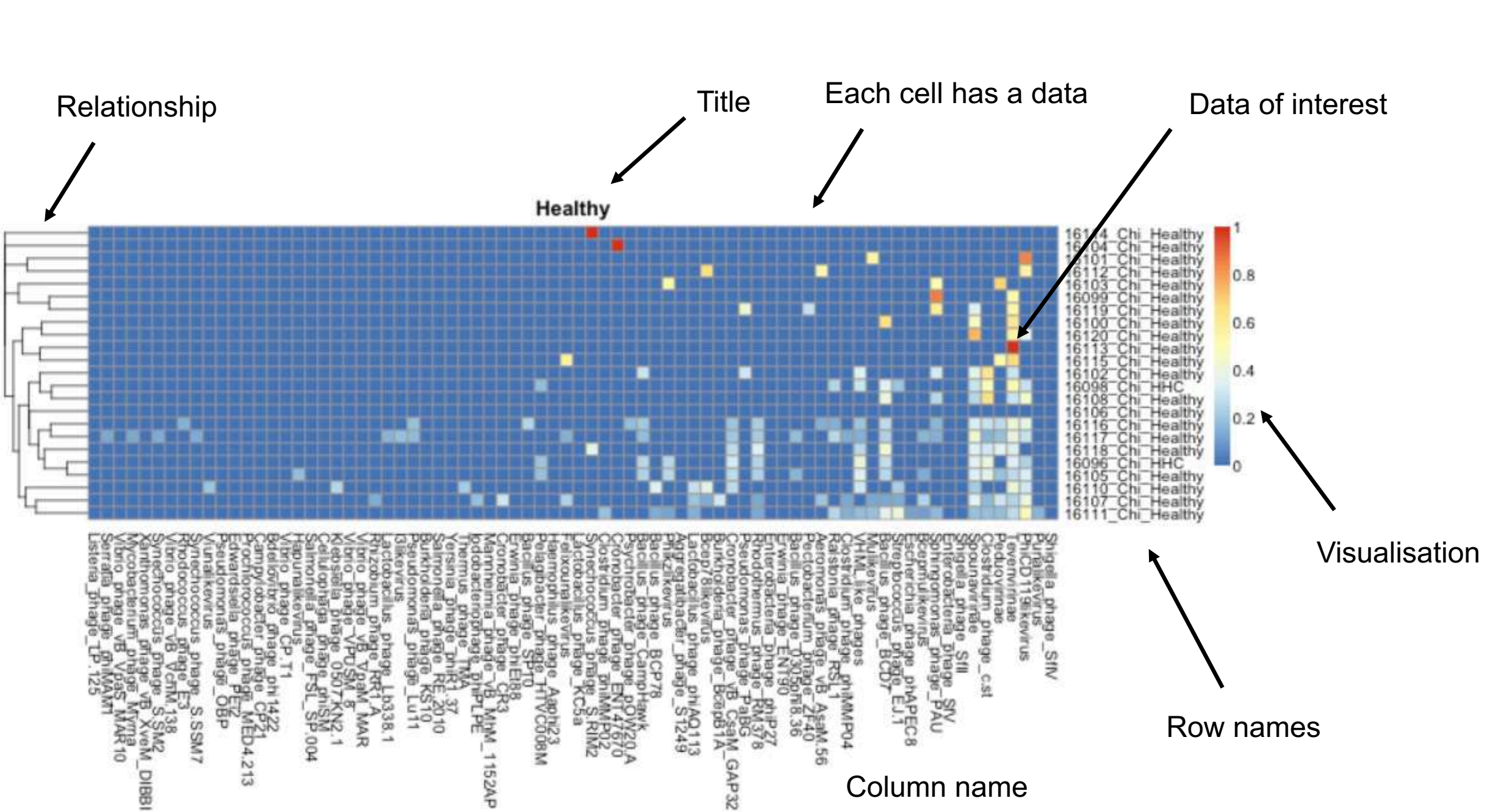


x10-30

When most people think of analysis

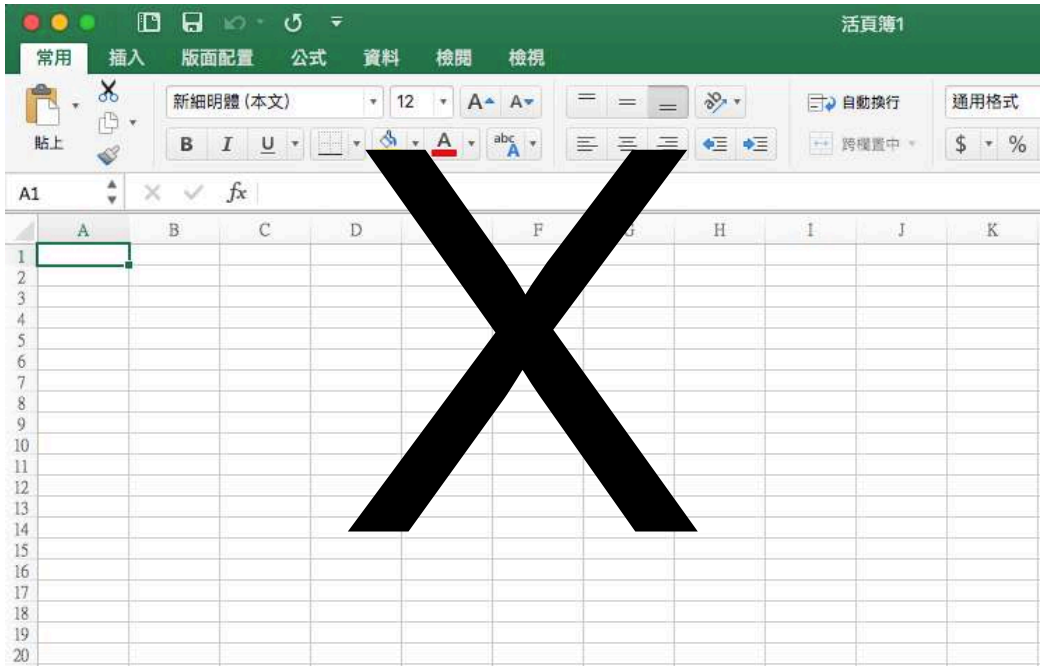


	A	B	C	D	E	F	G	H	I	J	K
1		Listeria_phag	Serratia_phag	Vibrio_phage	Mycobacteriu	Xanthomonas	Synechococcu	Vibrio_phage	Rhodococcus	Synechococcu	Viunalikeviru
2	16096_Chi_HHC	0	0	0	0	0	0	0	0	0	0
3	16098_Chi_HHC	0	0	0	0	0	0	0	0	0	0
4	16099_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
5	16100_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
6	16101_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
7	16102_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
8	16103_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
9	16104_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
10	16105_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
11	16106_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
12	16107_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
13	16108_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
14	16110_Chi_Healthy	0	0	0	0	0	0	0	0	0	26
15	16111_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
16	16112_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
17	16113_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
18	16114_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
19	16115_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
20	16116_Chi_Healthy	0	0	0	0	0	0	0	23	0	0
21	16117_Chi_Healthy	0	20	0	21	0	22	0	0	23	0
22	16118_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
23	16119_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
24	16120_Chi_Healthy	0	0	0	0	0	0	0	0	0	0


Relationship
Title
Each cell has a data
Data of interest
Healthy
1
0.8
0.6
0.4
0.2
0
Visualisation
Row names
Column name

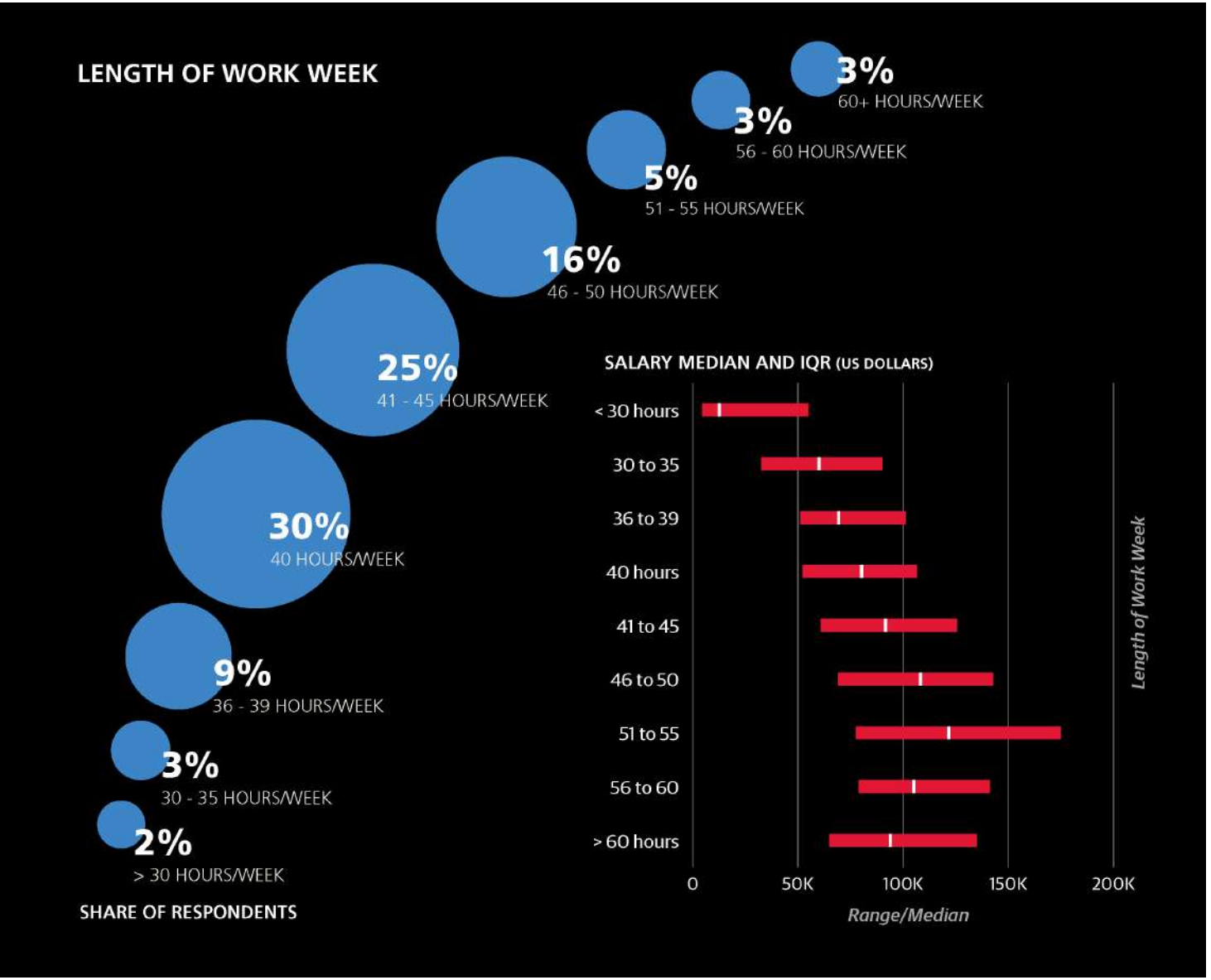
So what do you need?

You need a platform to rearrange, tidy, subset, merge data easily



Recommendation:
R and Python in a linux environment

2016 Data science survey



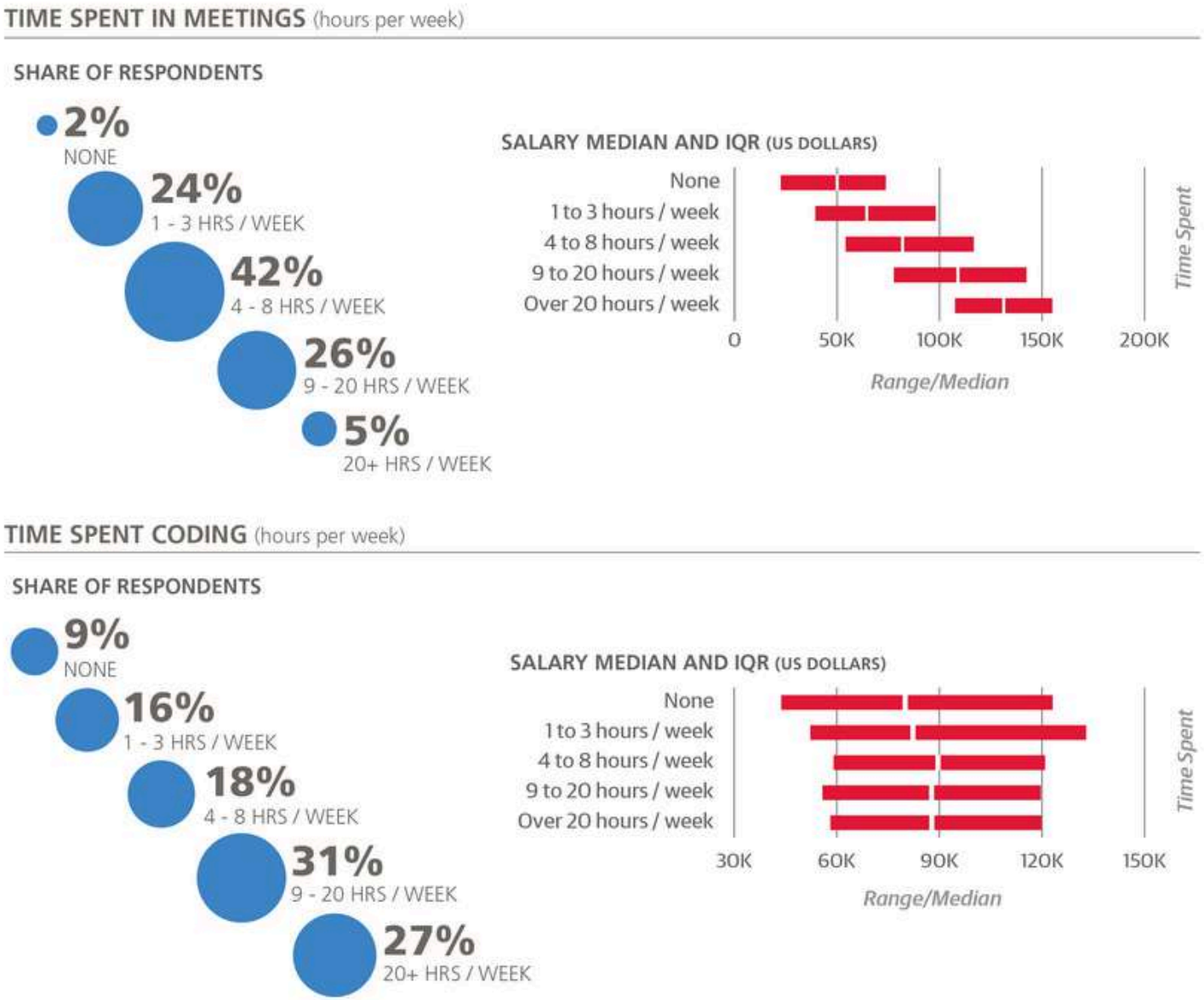
https://www.oreilly.com/ideas/2016-data-science-salary-survey-results

In Taiwan

40 hour = Monday - Friday
9am-6pm
one hour lunch break

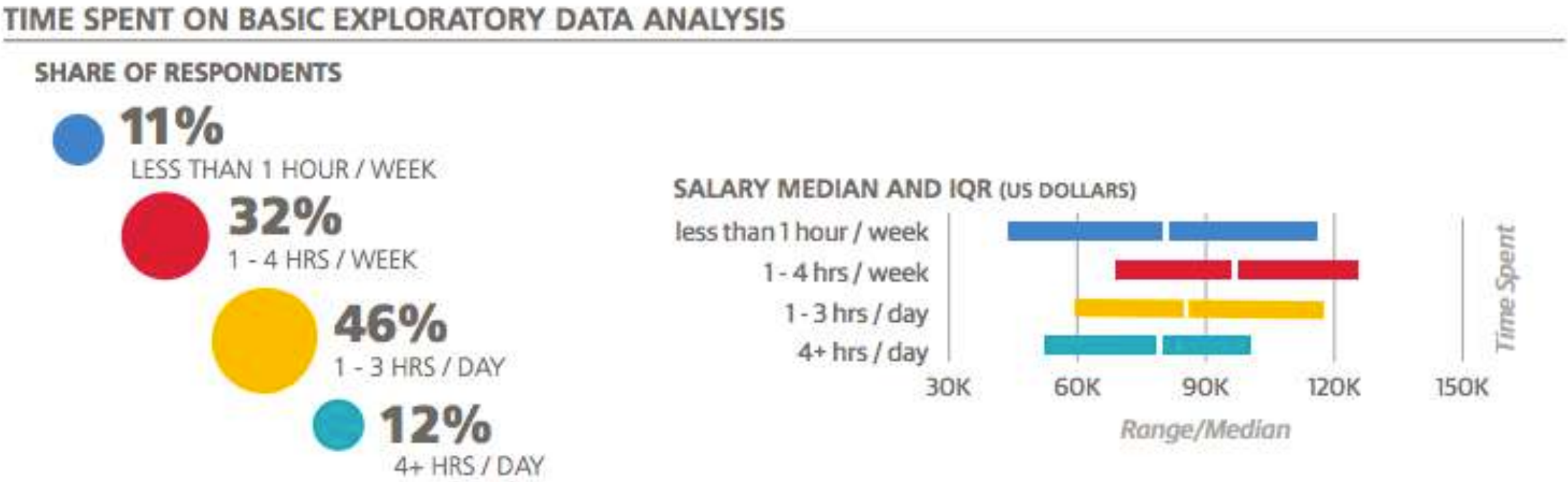
How much do you work a week?

Time spent in meetings and coding



https://www.oreilly.com/ideas/2016-data-science-salary-survey-results

2015 Data science survey



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2015 Data science survey

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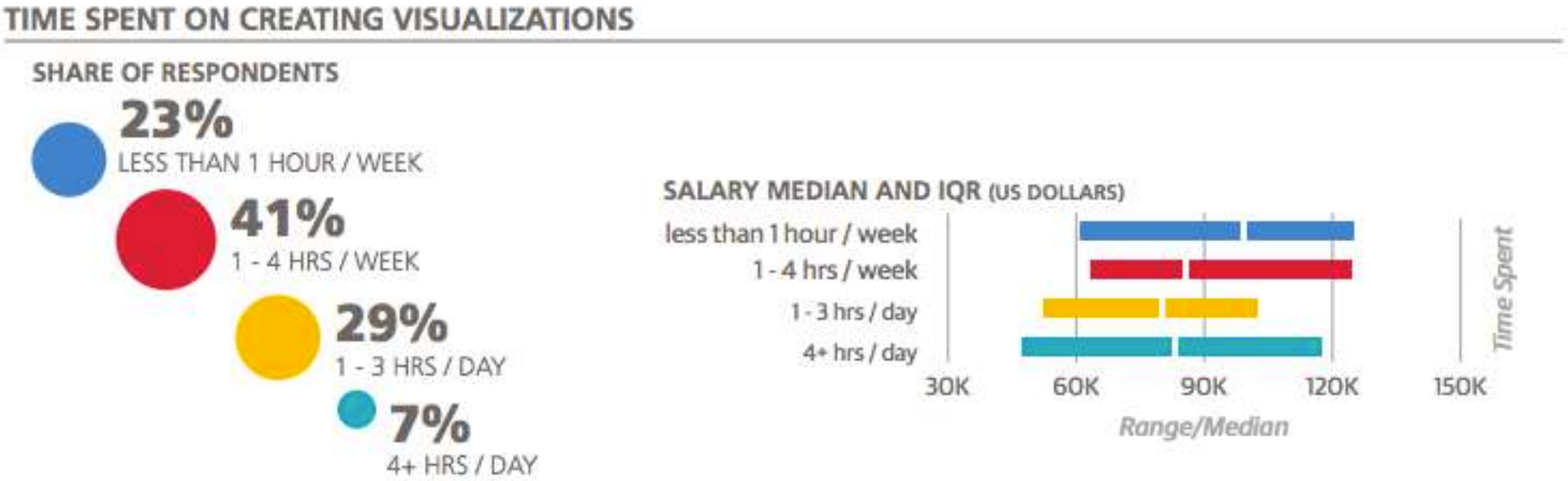
2015 Data science survey



https://www.oreilly.com/ideas/2015-data-science-salary-survey

Some observations

A day of a data scientist /bioinformatician / biologist with lots of data:

- **Less than 1 to 4 hours** to quickly explore data (78%)
- **Less than 1 to 4 hours** to do data cleaning (74%)
- **Less than 1 to 4 hours** to visualise data (70%)
- **Less than 1 to 4+ hours** to present analysis (73%)

= 4 – 16 hours to finish your daily task

Interview

Sarah Teichmann: 'I wake as early as 4am and think about work'

By Interview: Rosanna Greenstreet

The 42-year-old scientist is head of cellular genetics at the Wellcome Sanger Institute, Cambridge

Sleep I need seven or eight hours. My daughters, aged 10 and five, are in bed by 8.30pm. My husband and I have different methods of getting them to bed: he likes nature television programmes; I like reading in German. Both my father and husband are German, so we try to maintain the language. Before I go to sleep, I read books such as Sheryl Sandberg's Lean In, or essays from Harvard Business Review. I am usually asleep by nine and wake as early as 4am; it gives me a few hours to think about work before the rest of the family wakes at 7am.

Work There's a difference between how many hours you work and how many hours you are "at work". I am at work from 8.15am to 6pm and a lot of that time is spent in meetings. At weekends I work four or five hours around the family's schedule. As well as being head of a programme in Cambridge, I coordinate the Human Cell Atlas consortium, an international project to map all the cells in the human body, which involves a lot of travel.

https://www.theguardian.com/money/2018/mar/03/sarah-teichmann-wake-4am-think-about-work

Some observations (my own opinions)

- Data scientist are needed everywhere
- Bioinformatician / data scientist in Biology field are less well-paid in relative to other field,

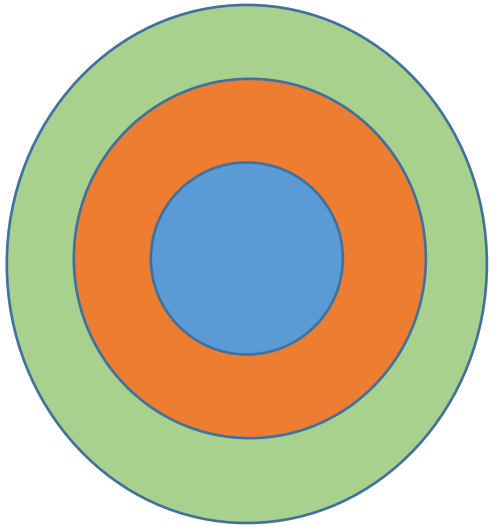
This will result in

- All high throughput data / analysis are outsourced to companies -> students/labs will not gain the experience
- A few labs can enjoy deal with all the data in Taiwan -> also not good as no energy to initiate novel projects

- Try to be as much hands on as possible early in your training

Linux

History of Unix



Unix is a family of multitasking, multiuser computer operating systems that derive from the original AT&T Unix, development starting in the 1970s at the Bell Labs research center by Ken Thompson, Dennis Ritchie, and others.

User

↕

Shell (execute commands from terminal window)

↕

Kernel (manages hardware resources)

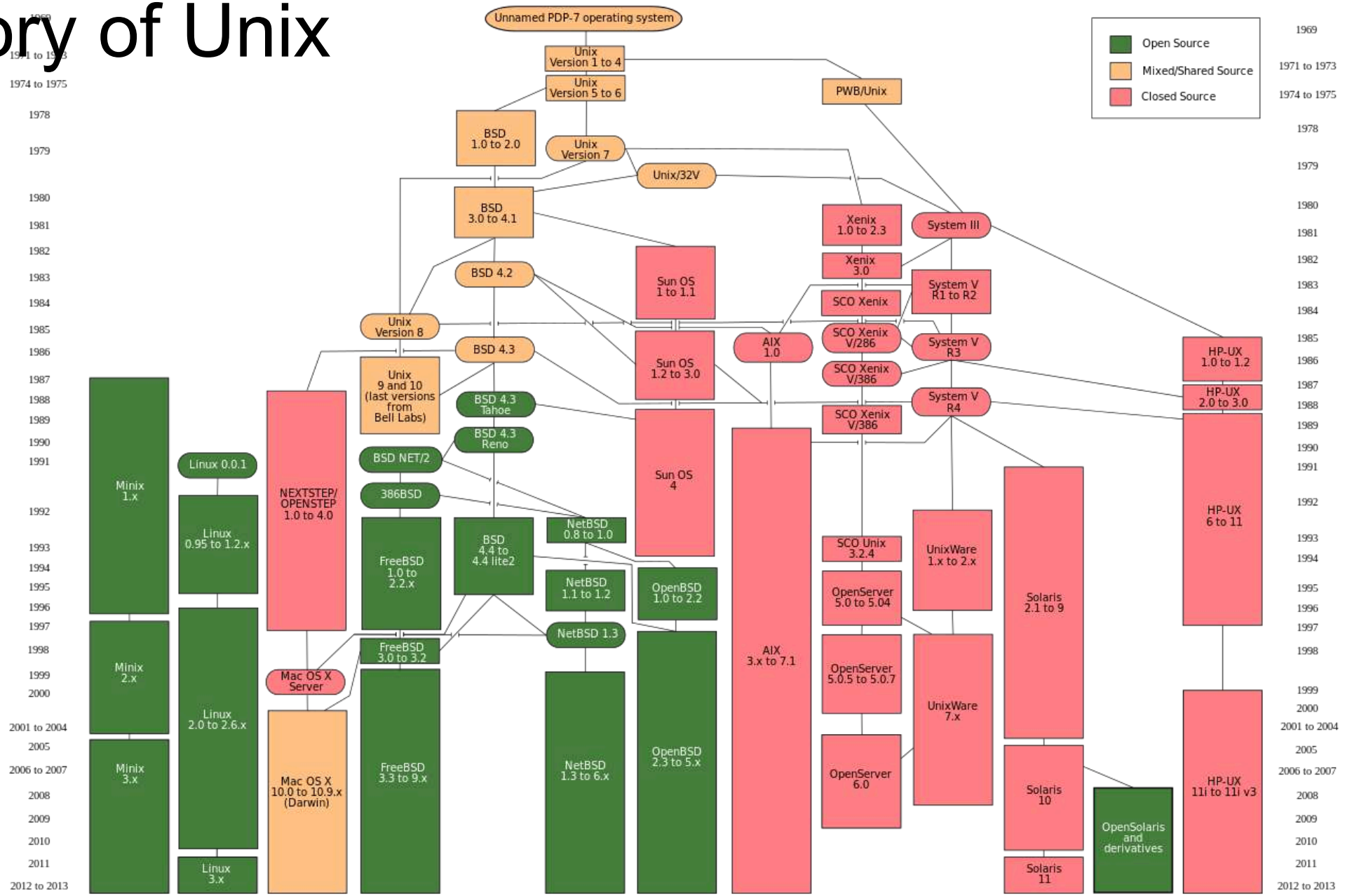
↕

Hardware (CPU, RAM, network…)

https://en.wikipedia.org/wiki/History_of_Unix

Wiki

History of Unix

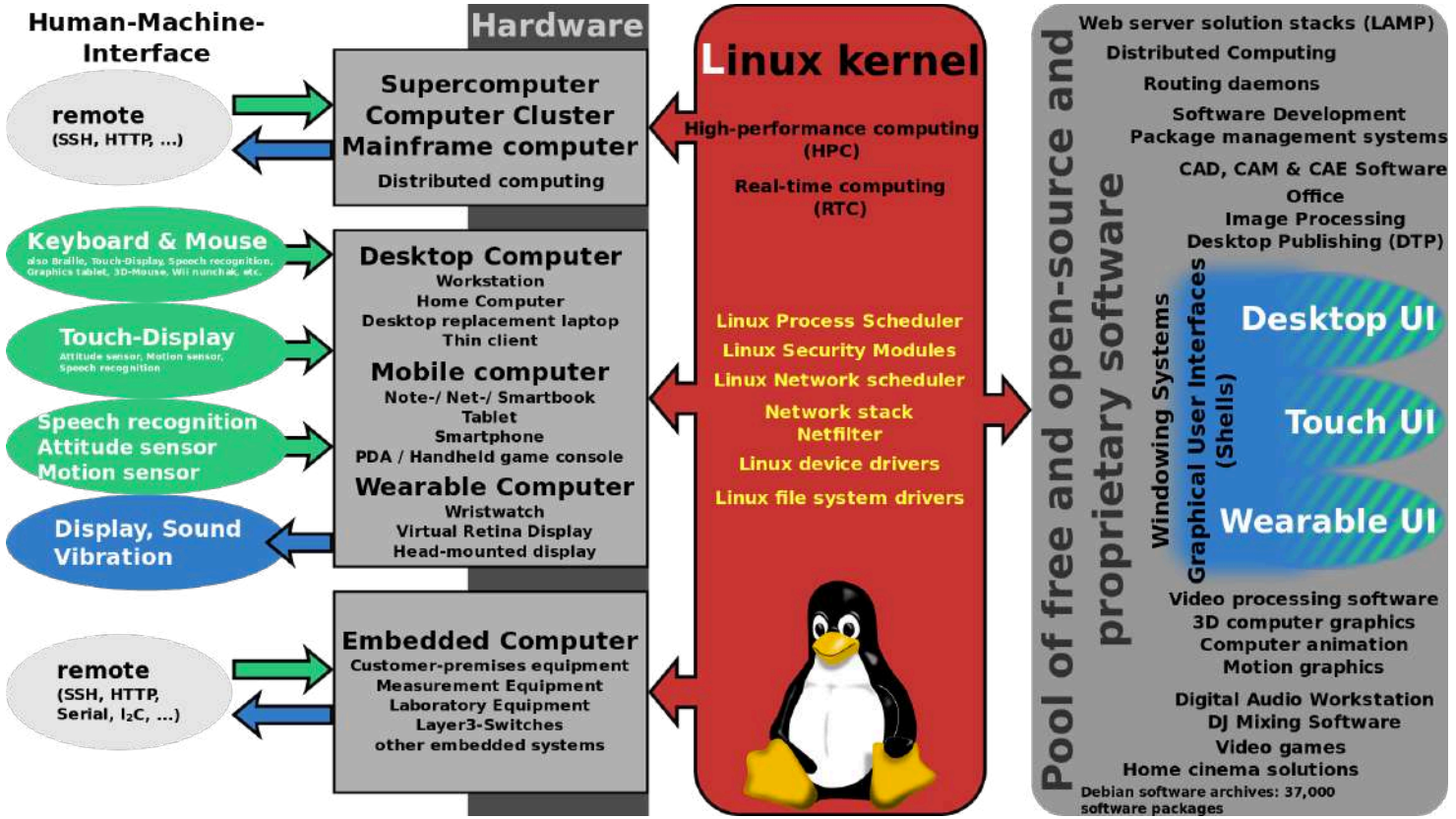


https://en.wikipedia.org/wiki/History_of_Unix

Wiki

What is GNU/Linux?

GNU/Linux is a **Unix-like** computer **operating system (OS)** assembled under the model of **free** and **open-source software** development and distribution.



Linux kernel was designed by Linux Torvalds
GNU project contains lots of UNIX-like libraries and applications

Wiki

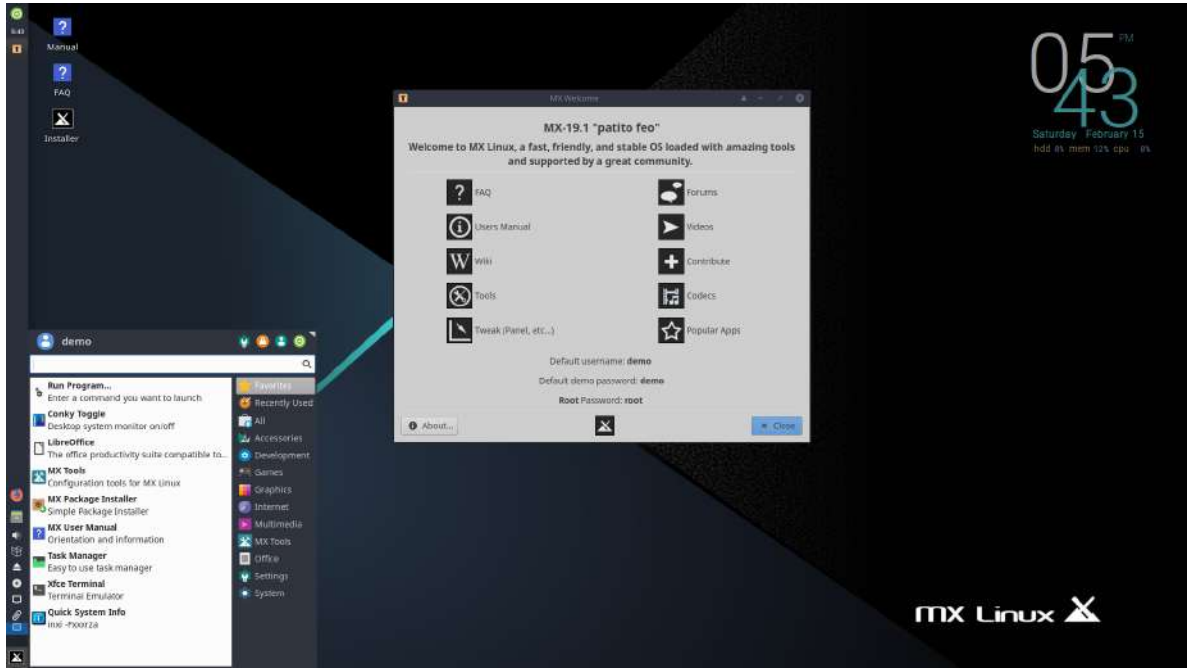
Linux distributions

A **Linux distribution** (often called a distro for short) is an operating system made from **a software collection**, which is based upon the Linux kernel and, often, a package management system.

資料範圍: Last 6 months 出發		
名次	發行版	HPD*
1	MX Linux	4704▼
2	Manjaro	2867▲
3	Mint	2365▲
4	Debian	1692▲
5	Ubuntu	1566▲
6	elementary	1407▲
7	Solus	1212▲
8	Fedora	1017▲
9	Zorin	995▲
10	deepin	941▲
11	KDE neon	812▲
12	antiX	810▲
13	CentOS	796▲
14	PCLinuxOS	740▲
15	ArcoLinux	735–
16	Pop!_OS	725▲
17	openSUSE	696–
18	Arch	691▲
19	Kali	577▲
20	Puppy	456–

Wiki

Linux distributions



Mx Linux

Mint

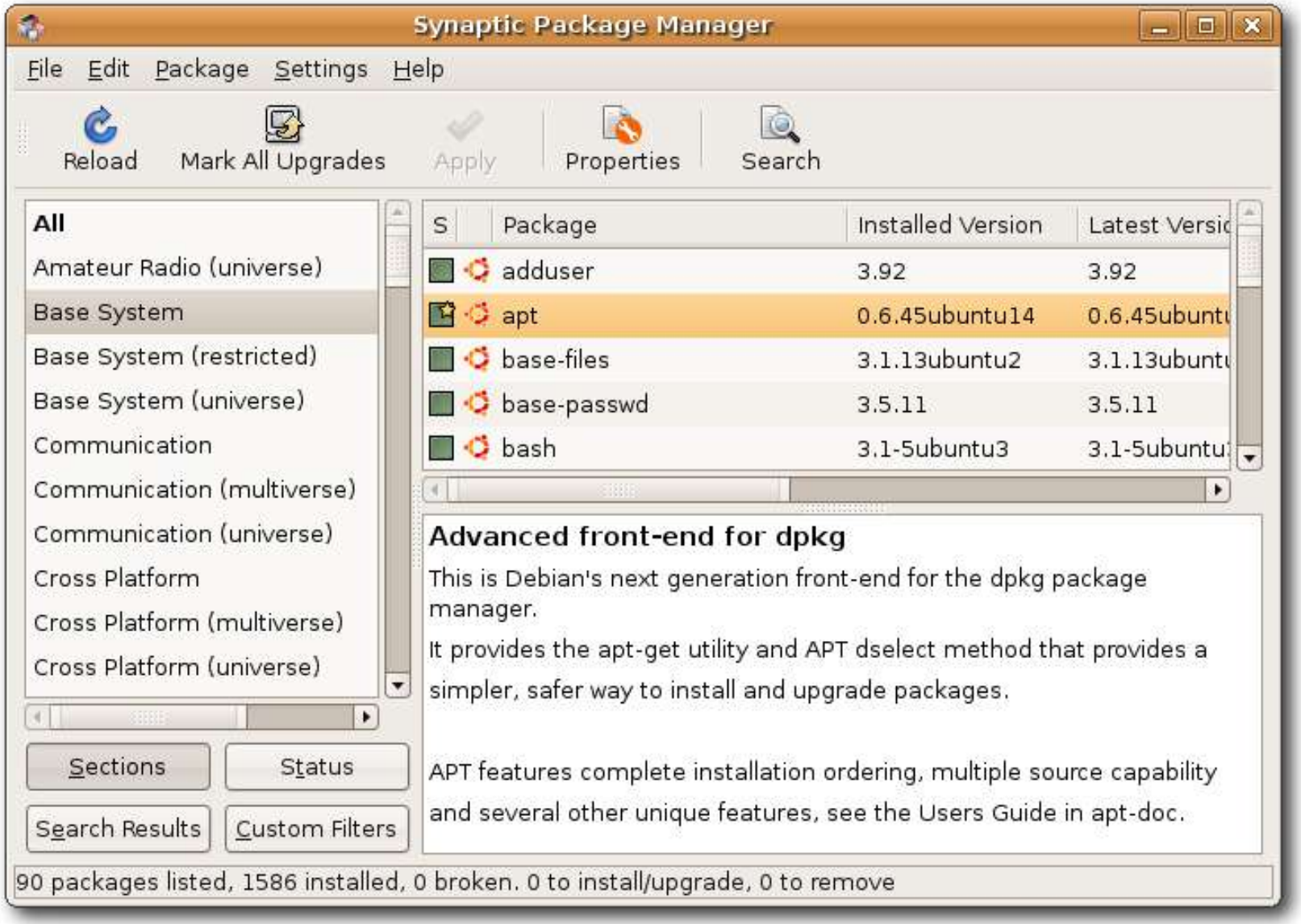
Installing programs in Linux

- Open-source and free
- More than one way of installing
  - From downloaded files
    - Binaries (already executable)
    - Compile from source files
  - From package manager (like App store)
    - Contains official repositories (secure, stable malware-free)
    - New repositories can be added (latest)

- Dependencies
  - A software uses (depends) another software which performs specific tasks

Installing programs in Linux

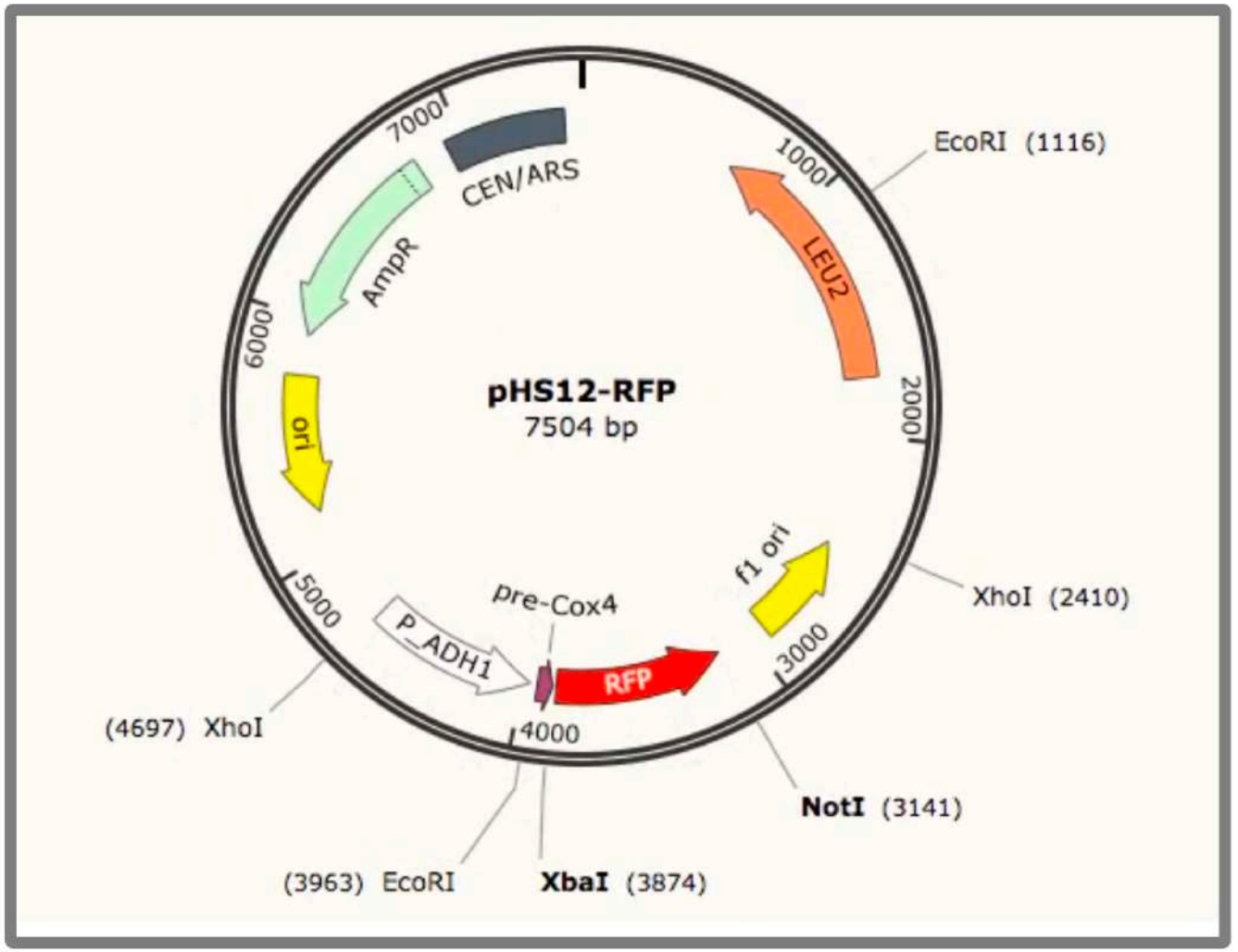
Desktop type
Software manager



https://help.ubuntu.com/community/SynapticHowto

Download files from internet

Would you like to move beyond hand-drawn plasmid maps?



SnapGene Viewer is revolutionary software that allows molecular biologists to create, browse, and share richly annotated DNA sequence files up to 1 Gbp in length.

https://www.snapgene.com/snapgene-viewer/

Download

- Windows
- macOS
- Ubuntu
- Fedora / Red Hat

System Requirements

OS	Windows 7 or later macOS 10.10 or later Fedora Linux 21 or later Red Hat Linux 7.2 or later Ubuntu Linux 14.04 or later
Memory	1 GB RAM
Hard Disk	250 MB available disk space
Display	1024 x 768 or higher resolution

Download files from internet (II)

- **Support for complex barcodes, e.g. inDrop:**
  - Complex barcodes in STARsolo with --soloType CB_UMI_Complex, --soloCBmatchWLtype --soloAdapterSequence, --soloAdapterMismatchesNmax, --soloCBposition,--soloUMIposition
- **BAM tags:**
  - CB/UB for corrected CellBarcode/UMI
  - GX/GN for gene ID/name
- STARsolo most up-to-date documentation.

▼ Assets 2

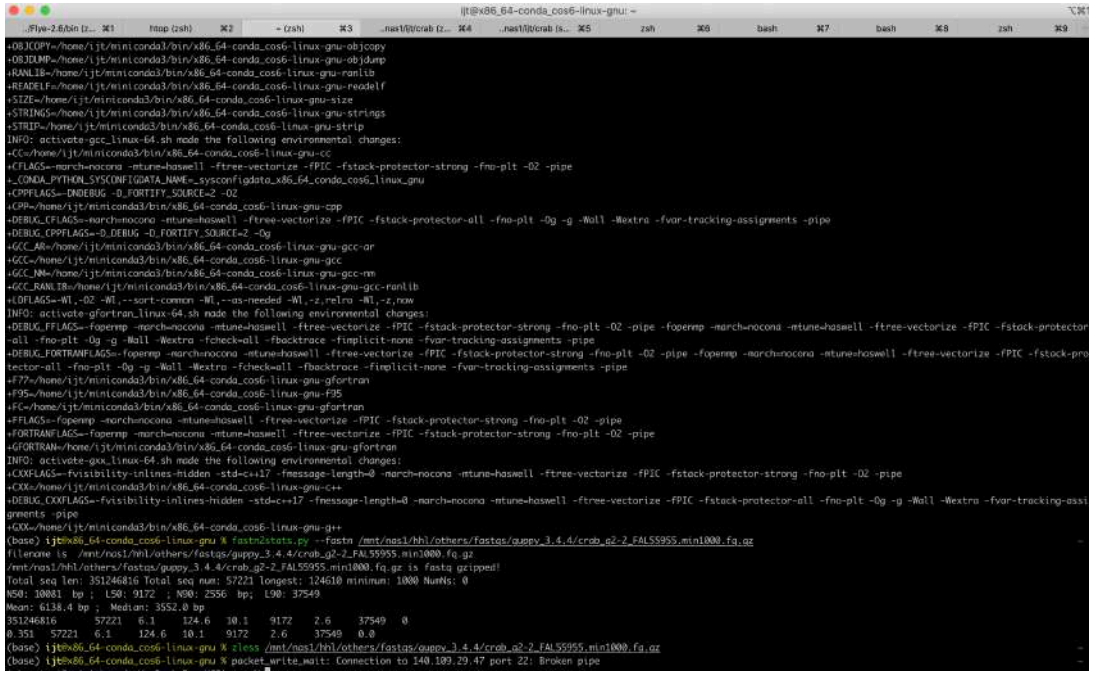
Source code (zip)

Source code (tar.gz)

Source code
Some may contain executable binaries
Some need to be compiled from scratch
Usually come in compressed file (need to decompress them)

https://www.snapgene.com/snapgene-viewer/

Console and Command-line interface



Console and Command-line interface

Computer terminal or system consoles are the **text entry and display device** for system administration messages, particularly those from the BIOS or boot loader, the kernel, from the init system and from the system logger. It is a **physical device consisting of a keyboard and a screen**.

A **command-line interface** is a means of interacting with a computer program where the **user** issues **commands** to the program (putty, terminal) in the form of successive lines of text (command lines).

http://www.ictlounge.com/html/operating_systems.htm Wiki

Using command line in day-to-day bioinformatics

- Most sequence files are text files
- Text mining easy!

- Features programming functions (e.g., loops, variables)
- Lots of little scripts

- Package everything (scripts, programs) into working pipelines

- Automation and reproducibility

- Remote access

A typical command

Options always start with ‘-’, and often expect to receive an option (xxx)

Special characters in bash

CHARACTER	MEANING
SPACE	Separate commands and arguments
# POUND	Comment
; SEMICOLON	Command separator two run multiple commands
. DOT	Source command OR filename component OR current directory
.. DOUBLE DOTS	Parent directory
'' SINGLE QUOTES	Use expression between quotes literaly
, COMMA	Concatenate strings
\ BACKSLASH	Escape for single character
/ SLASH	Filename path separator
* ASTERISK	Wild card for filename expansion in globbing
>, <, >> CHARACTERS	Redirection input/outputs
| PIPE	Pipe outputs between commands

Special characters in bash

```
$ command xxxx yyyyy
```

Linux treats xxxx and yyyy as two arguments of the command

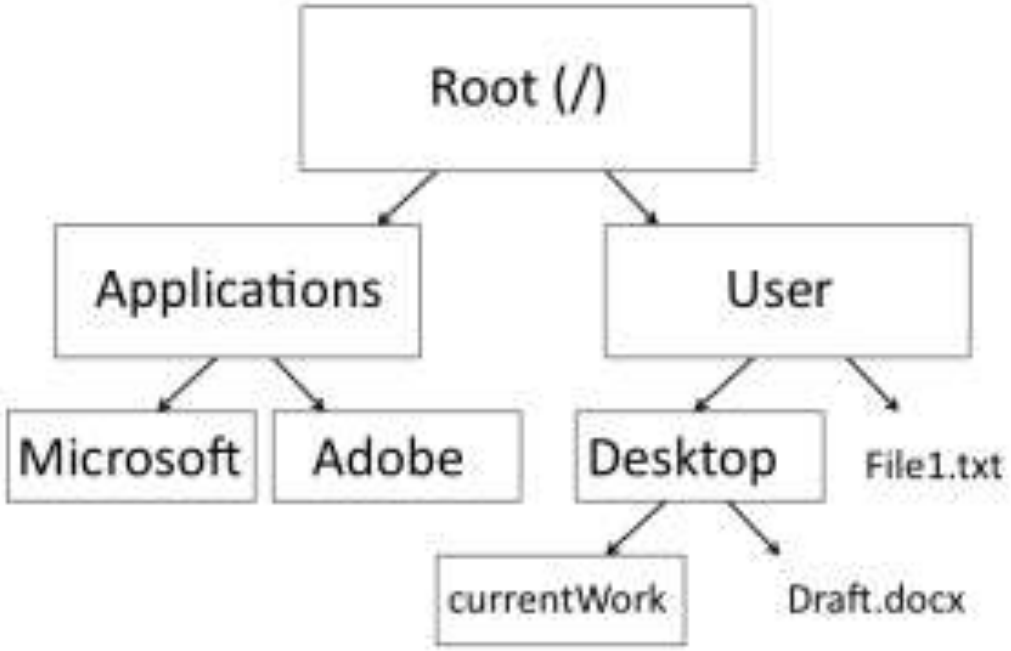
```
$ command ‘xxxx yyyyy’
$ command xxxx\ yyyy
```

You can uses single quotes or escape to distinguish special characters (in this case: space)

Short cut and emergency command in linux

SHORTCUT	MEANING
Tab	Autocomplete files or folder names
↑	Scroll up to the command history
↓	Scroll down to the command history
Ctrl + A	Go to the beginning of the line that you are typing
Ctrl + D	Go to the end of the line that you are typing
Ctrl + U	Clear all the line (or until the cursor position)
Ctrl + R	Search previously used commands
* Ctrl + C	Kill the process that you are running
Ctrl + D	Exit the current shell
Ctrl + Z	Put the running process to the background. Use command fg to recover it.

Directory structure



Try:
ls (list segment)
cd (change directory)
rm (abbreviation for remove)
mkdir (make directory)
pwd (print working directory)

Directory structure is like a tree

From /home/ishengtsai/

Relative path:

```
cd fungi        # moves into fungi folder
                # now you are in /home/ishengtsai/fungi/
                # you can only do this successfully when you are in /home/ishengtsai/

cd ..           # you go up one directory
                # now you are in /home/
```

Or absolute path:

```
cd /home/ishengtsai/fungi/ ;
```

Files commands **

COMMAND	USE	EXAMPLE
less	Open a file with less. Q to exit. Arrows to scroll	less myfile
touch	Create an empty file	touch myfile
mv	Move file between dirs. Change name	mv myfile yourfile
rm	Remove file	rm youfil
cat	Print file content as STDOUT	cat myfile
head	Print first 10 lines as STDOUT	head myfile
tail	Print last 10 lines as STDOUT	tail myfile
grep	Print matching lines as STDOUT	grep 'ATG' myfile
cut	Cut columns and print as STDOUT	cut -f1 myfile
sort	Sort lines and print as STDOUT	sort myfile
sed	Replace ocurrences, print lines STDOUT	sed 's/ATG/CTG/' myfile
wc	Word count	wc myfile

awk https://en.wikipedia.org/wiki/AWK

Compression commands

COMMAND	USE	EXAMPLE
gzip	Compress a file using gzip	gzip -c test.txt > test.txt.gz
gunzip	Uncompress a file using gzip	gunzip test.txt.gz
bzip2	Compress a file using bzip	bzip2 -c test.txt > test.txt.bz2
bunzip2	Uncompress a file using gzip	bunzip2 test.txt.bz2
tar	Archive files usint tar	tar -cf sample.tar sample/*.txt
tar -zcvf	Archive using tar and compress using gzip	tar -zcvf samples.tar.gz sample/*.txt
tar -zxvf	Unarchive using tar and uncompress using gunzip	tar -zxvf samples.tar.gz
tar -jcvf	Archive using tar and compress using bzip2	tar -jcvf samples.tar.bz2 sample/*.txt
tar -jxvf	Unarchive using tar and uncompress using bunzip2	tar -jxvf samples.tar.bz2

Redirection of input / output

The result of the **ls** command will be output and saved into **out.txt**

```
$ ls > out.txt
```

The result of the **ls** command will be output and **append** into **out.txt**
If the file **out.txt** already exists, then the original content will not be **replaced**, and
the new information will be added into the file

```
$ ls >> out.txt
```

Pipeline

… a **pipeline** is a set of **processes** chained by their **standard streams**, so that the output of each process (stdout) feeds directly as input (stdin) to the next one.

program1 | program2 | program3

Special character to **pipe** the results

Example:

ls -l | grep key | less

Demonstration I: daily tasks

1. Login into a terminal
2. Go to a specific directory that contains your data
3. Inspect your **fasta** files
   $ less ref.fa | grep '>' | less
   $ less ref.fa | grep '>' | wc -l

4. How about **fastq** file?
   - how many sequences?

5. How about gff file?
   - how many exons? How many genes?
   - how many genes that are expressed in the forward strand?

6. Check if command is successful

Installation

1. You need a bioinformatics program
    1. Download binaries and it should be ready to execute
    2. Or you have to compile
    3. Most modern program now deposit their program in **github**

```
cd /home/ijt/NGScourse/
git clone https://github.com/relipmoc/skewer.git
cd skewer
make
/home/ijt/NGScourse/skewer/skewer
```

compile

Ready to run!

Jiang et al. BMC Bioinformatics 2014, 15:182
http://www.biomedcentral.com/1471-2105/15/182

BMC Bioinformatics

METHODOLOGY ARTICLE Open Access

Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads

Hongshan Jiang[1*], Rong Lei[1], Shou-Wei Ding[2] and Shuifang Zhu[1]

Demonstration II: daily tasks

1. Downloaded some sequenced data ; mapped to genome and you want to start looking at it.

2. Look at sam file
   $ samtools view xxx.bam | less

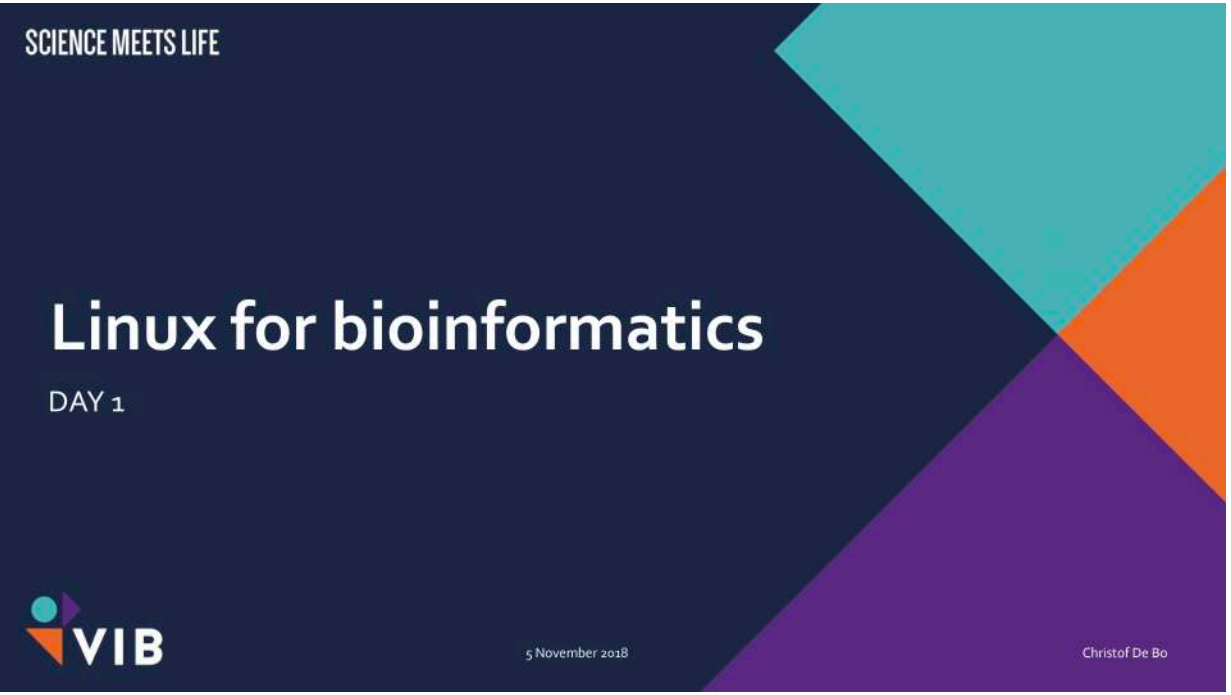
3. Okay, how about if I want to check the insert size of properly mapped reads? What filter to use? (https://broadinstitute.github.io/picard/explain-flags.html)

4. You have a file that you want to visualize, what next?

Demonstration III: daily tasks

1. How many genes are there in a gff file?
2. Length of fasta files
3. Longest sequence in the fasta (if not sorted)
4. Scripts to find out
5. Echo
6. For loop

Good references

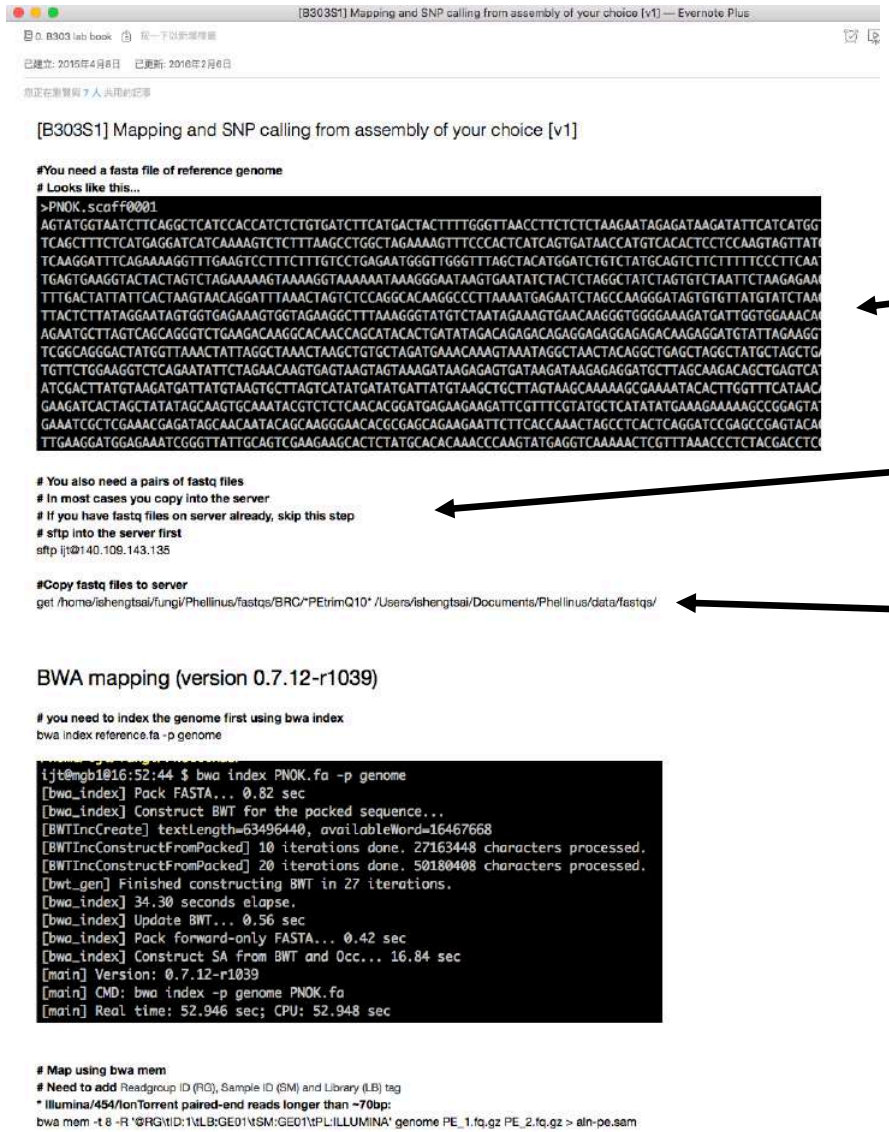


https://www.bits.vib.be/training-list/112-bits/training/upcoming-trainings/124-linux-for-bioinformatics

Keep tracking

Keep a track of your science

Evernote; onenote; notion.. Etc?



[B303S1] Mapping and SNP calling from assembly of your choice [v1] — Evernote Plus

0. B303 lab book

已建立: 2015年4月8日 已更新: 2016年2月6日

[B303S1] Mapping and SNP calling from assembly of your choice [v1]

#You need a fasta file of reference genome
 # Looks like this...

```
>PNOK.scaff0001
AGTATGGTAATCTTCAGGCTCATCCACCATCTCTGTGATCTTCATGACTACTTTTGGGTTAACCTTCTCTCTAAGAATAGAGATAAGATATTCATCATGG
TCAGCTTTCTCATGAGGATCATCAAAAGTCTCTTTAAGCCTGGCTAGAAAAGTTTCCCACTCATCAGTGATAACCATGTCACACTCCTCCAAGTAGTTAT
TCAAGGATTTCAGAAAAGGTTTGAAGTCCTTTCTTTGTCCTGAGAATGGGTTGGGTTTAGCTACATGGATCTGTCTATGCAGTCTTCTTTTTCCCTTCAA
TGAGTGAAGGTACTACTAGTCTAGAAAAAGTAAAAGGTAAAAAATAAAGGGAATAAGTGAATATCTACTCTAGGCTATCTAGTGTCTAATTCTAAGAGAA
TTTGACTATTATTCACTAAGTAACAGGATTTAAACTAGTCTCCAGGCACAAGGCCCTTAAAATGAGAATCTAGCCAAGGGATAGTGTGTTATGTATCTAA
TTACTCTTATAGGAATAGTGGTGAGAAAGTGGTAGAAGGCTTTAAAGGGTATGTCTAATAGAAAGTGAACAAGGGTGGGGAAAGATGATTGGTGGAAACA
AGAATGCTTAGTCAGCAGGGTCTGAAGACAAGGCACAACCAGCATACACTGATATAGACAGAGACAGAGGAGAGGAGACAAGAGGATGTATTAGAAGG
TCGGCAGGGACTATGGTTAAACTATTAGGCTAAACTAAGCTGTGCTAGATGAAACAAAGTAAATAGGCTAACTACAGGCTGAGCTAGGCTATGCTAGCTG
TGTTCTGGAAGGTCTCAGAATATTCTAGAACAAGTGAGTAAGTAGTAAAGATAAGAGAGTGATAAGATAAGAGAGGATGCTTAGCAAGACAGCTGAGTCA
ATCGACTTATGTAAGATGATTATGTAAGTGCTTAGTCATATGATATGATTATGTAAGCTGCTTAGTAAGCAAAAAGCGAAAATACACTTGGTTTCATAAC
GAAGATCACTAGCTATATAGCAAGTGCAAATACGTCTCTCAACACGGATGAGAAGAAGATTCGTTTCGTATGCTCATATATGAAAGAAAAAGCCGGAGTA
GAAATCGCTCGAAACGAGATAGCAACAATACAGCAAGGGAACACGCGAGCAGAAGAATTCTTCACCAAACTAGCCTCACTCAGGATCCGAGCCGAGTACA
TTGAAGGATGGAGAAATCGGGTTATTGCAGTCGAAGAAGCACTCTATGCACACAAACCCAAGTATGAGGTCAAAAACTCGTTTAAACCCTCTACGACCTC
```

 # You also need a pairs of fastq files
 # In most cases you copy into the server
 # If you have fastq files on server already, skip this step
 # sftp into the server first
sftp ijt@140.109.143.135

#Copy fastq files to server
get /home/ishengtsai/fungi/Phellinus/fastqs/BRC/*PEtrimQ10* /Users/ishengtsai/Documents/Phellinus/data/fastqs/

BWA mapping (version 0.7.12-r1039)

 # you need to index the genome first using bwa index
bwa index reference.fa -p genome

```
ijt@mgb1016:52:44 $ bwa index PNOK.fa -p genome
[bwa_index] Pack FASTA... 0.82 sec
[bwa_index] Construct BWT for the packed sequence...
[BWTIncCreate] textLength=63496440, availableWord=16467668
[BWTIncConstructFromPacked] 10 iterations done. 27163448 characters processed.
[BWTIncConstructFromPacked] 20 iterations done. 50180408 characters processed.
[bwt_gen] Finished constructing BWT in 27 iterations.
[bwa_index] 34.30 seconds elapse.
[bwa_index] Update BWT... 0.56 sec
[bwa_index] Pack forward-only FASTA... 0.42 sec
[bwa_index] Construct SA from BWT and Occ... 16.84 sec
[main] Version: 0.7.12-r1039
[main] CMD: bwa index -p genome PNOK.fa
[main] Real time: 52.946 sec; CPU: 52.948 sec
```

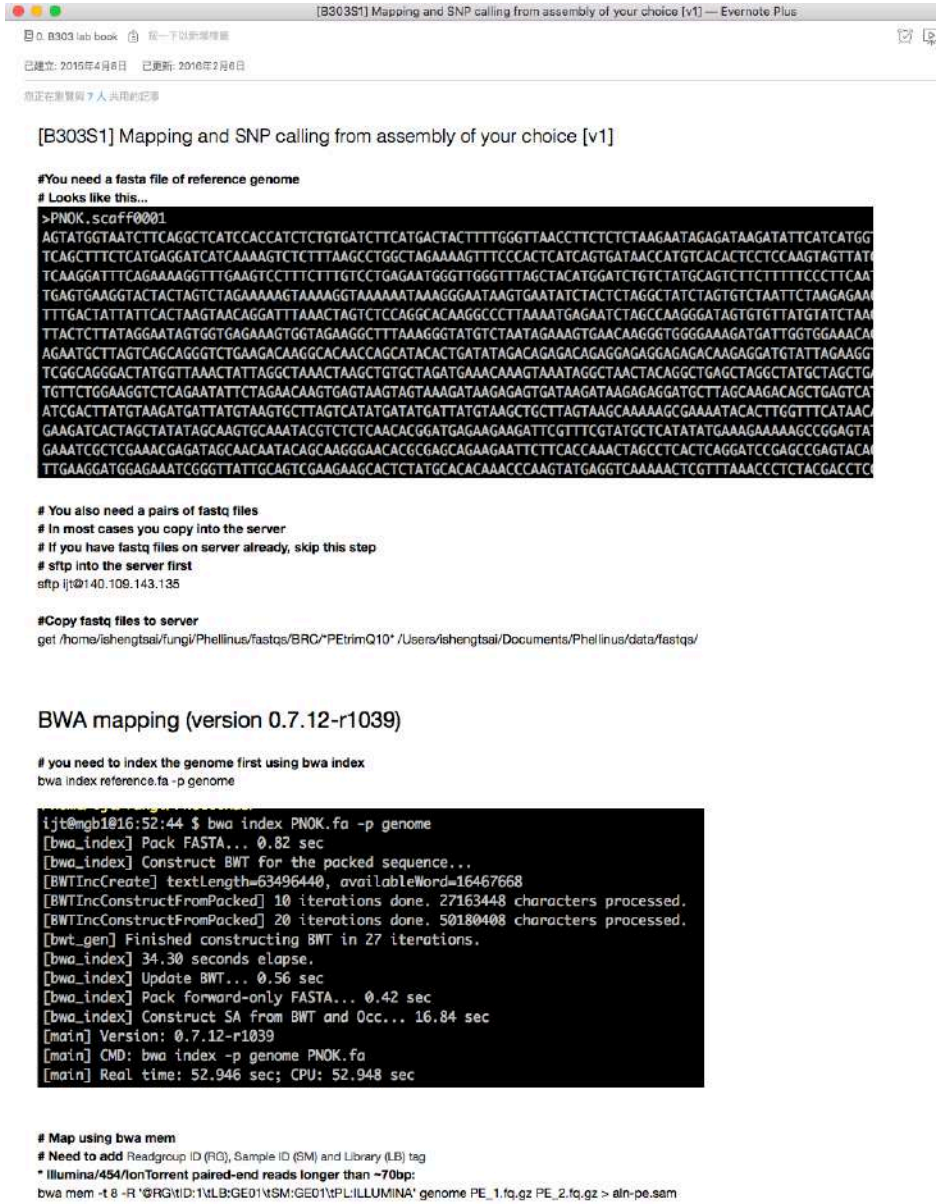
 # Map using bwa mem
 # Need to add Readgroup ID (RG), Sample ID (SM) and Library (LB) tag
*** Illumina/454/IonTorrent paired-end reads longer than ~70bp:**
bwa mem -t 8 -R '@RG\tID:1\tLB:GE01\tSM:GE01\tPL:ILLUMINA' genome PE_1.fq.gz PE_2.fq.gz > aln-pe.sam

Screenshot to log results

Comment your code (what was the purpose)

All the command can be reused (copy and paste!)

Evernote / Notion



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0. B303 lab book

已建立: 2015年4月8日 已更新: 2016年2月8日

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TCAGCTTTCTCATGAGGATCATCAAAAGTCTCTTTAAGCCTGGCTAGAAAAGTTTCCCACTCATCAGTGATAACCATGTCACACTCCTCCAAGTAGTTAT
TCAAGGATTTCAGAAAAGGTTTGAAGTCCTTTCTTTGTCCTGAGAATGGGTTGGGTTTAGCTACATGGATCTGTCTATGCAGTCTTCTTTTTCCCTTCAA
TGAGTGAAGGTACTACTAGTCTAGAAAAAGTAAAAGGTAAAAAATAAAGGGAATAAGTGAATATCTACTCTAGGCTATCTAGTGTCTAATTCTAAGAGAA
TTTGACTATTATTCACTAAGTAACAGGATTTAAACTAGTCTCCAGGCACAAGGCCCTTAAAATGAGAATCTAGCCAAGGGATAGTGTGTTATGTATCTAA
TTACTCTTATAGGAATAGTGGTGAGAAAGTGGTAGAAGGCTTTAAAGGGTATGTCTAATAGAAAGTGAACAAGGGTGGGGAAAGATGATTGGTGGAAACA
AGAATGCTTAGTCAGCAGGGTCTGAAGACAAGGCACAACCAGCATACACTGATATAGACAGAGACAGAGGAGAGGAGACAAGAGGATGTATTAGAAGG
TCGGCAGGGACTATGGTTAAACTATTAGGCTAAACTAAGCTGTGCTAGATGAAACAAAGTAAATAGGCTAACTACAGGCTGAGCTAGGCTATGCTAGCTG
TGTTCTGGAAGGTCTCAGAATATTCTAGAACAAGTGAGTAAGTAGTAAAGATAAGAGAGTGATAAGATAAGAGAGGATGCTTAGCAAGACAGCTGAGTCAT
ATCGACTTATGTAAGATGATTATGTAAGTGCTTAGTCATATGATATGATTATGTAAGCTGCTTAGTAAGCAAAAAGCGAAAATACACTTGGTTTCATAAC
GAAGATCACTAGCTATATAGCAAGTGCAAATACGTCTCTCAACACGGATGAGAAGAAGATTCGTTTCGTATGCTCATATATGAAAGAAAAAGCCGGAGTA
GAAATCGCTCGAAACGAGATAGCAACAATACAGCAAGGGAACACGCGAGCAGAAGAATTCTTCACCAAACTAGCCTCACTCAGGATCCGAGCCGAGTACA
TTGAAGGATGGAGAAATCGGGTTATTGCAGTCGAAGAAGCACTCTATGCACACAAACCCAAGTATGAGGTCAAAAACTCGTTTAAACCCTCTACGACCTC
```

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#Copy fastq files to server
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```
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[BWTIncConstructFromPacked] 10 iterations done. 27163448 characters processed.
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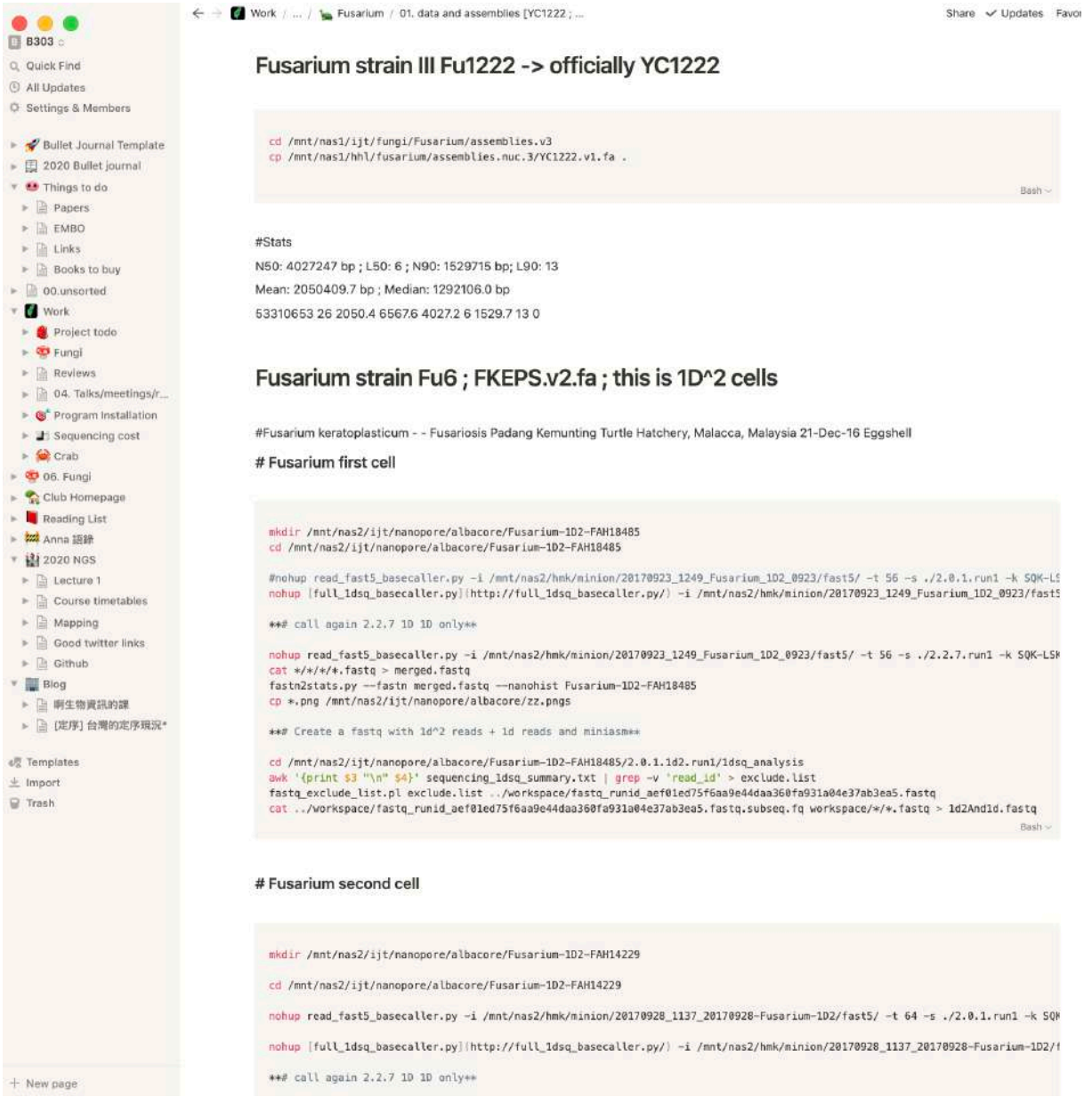
```

 # Map using bwa mem
 # Need to add Readgroup ID (RG), Sample ID (SM) and Library (LB) tag
*** Illumina/454/IonTorrent paired-end reads longer than ~70bp:**
bwa mem -t 8 -R '@RG\tID:1\tLB:GE01\tSM:GE01\tPL:ILLUMINA' genome PE_1.fq.gz PE_2.fq.gz > aln-pe.sam



B303

Quick Find
All Updates
Settings & Members

Bullet Journal Template
2020 Bullet journal
Things to do
Papers
EMBO
Links
Books to buy
00.unsorted
Work
Project todo
Fungi
Reviews
04. Talks/meetings/r...
Program Installation
Sequencing cost
Crab
06. Fungi
Club Homepage
Reading List
Anna 追錄
2020 NGS
Lecture 1
Course timetables
Mapping
Good twitter links
Github
Blog
啊生物資訊的課
[定序] 台灣的定序現況*

Templates
Import
Trash

New page

Work / ... / Fusarium / 01. data and assemblies [YC1222 ; ...

Share Updates Favor

Fusarium strain III Fu1222 -> officially YC1222

```
cd /mnt/nas1/ijt/fungi/Fusarium/assemblies.v3
cp /mnt/nas1/hhl/fusarium/assemblies.nuc.3/YC1222.v1.fa .
```

Bash

#Stats
N50: 4027247 bp ; L50: 6 ; N90: 1529715 bp; L90: 13
Mean: 2050409.7 bp ; Median: 1292106.0 bp
53310653 26 2050.4 6567.6 4027.2 6 1529.7 13 0

Fusarium strain Fu6 ; FKEPS.v2.fa ; this is 1D^2 cells

#Fusarium keratoplasticum - - Fusariosis Padang Kemunting Turtle Hatchery, Malacca, Malaysia 21-Dec-16 Eggshell

 # Fusarium first cell

```
mkdir /mnt/nas2/ijt/nanopore/albacore/Fusarium-1D2-FAH18485
cd /mnt/nas2/ijt/nanopore/albacore/Fusarium-1D2-FAH18485

#nohup read_fast5_basecaller.py -i /mnt/nas2/hmk/minion/20170923_1249_Fusarium_1D2_0923/fast5/ -t 56 -s ./2.0.1.run1 -k SQK-LS
nohup [full_1dsq_basecaller.py](http://full_1dsq_basecaller.py/) -i /mnt/nas2/hmk/minion/20170923_1249_Fusarium_1D2_0923/fast5

**# call again 2.2.7 1D 1D only**

nohup read_fast5_basecaller.py -i /mnt/nas2/hmk/minion/20170923_1249_Fusarium_1D2_0923/fast5/ -t 56 -s ./2.2.7.run1 -k SQK-LSK
cat */*/*/*.fastq > merged.fastq
fastn2stats.py --fastn merged.fastq --nanohist Fusarium-1D2-FAH18485
cp *.png /mnt/nas2/ijt/nanopore/albacore/zz.pngs

**# Create a fastq with 1d^2 reads + 1d reads and miniasm**

cd /mnt/nas2/ijt/nanopore/albacore/Fusarium-1D2-FAH18485/2.0.1.1d2.run1/1dsq_analysis
awk '{print $3 "\n" $4}' sequencing_1dsq_summary.txt | grep -v 'read_id' > exclude.list
fastq_exclude_list.pl exclude.list ../workspace/fastq_runid_aef01ed75f6aa9e44daa360fa931a04e37ab3ea5.fastq
cat ../workspace/fastq_runid_aef01ed75f6aa9e44daa360fa931a04e37ab3ea5.fastq.subseq.fq workspace/*/*.fastq > 1d2And1d.fastq
```

Bash

 # Fusarium second cell

```
mkdir /mnt/nas2/ijt/nanopore/albacore/Fusarium-1D2-FAH14229

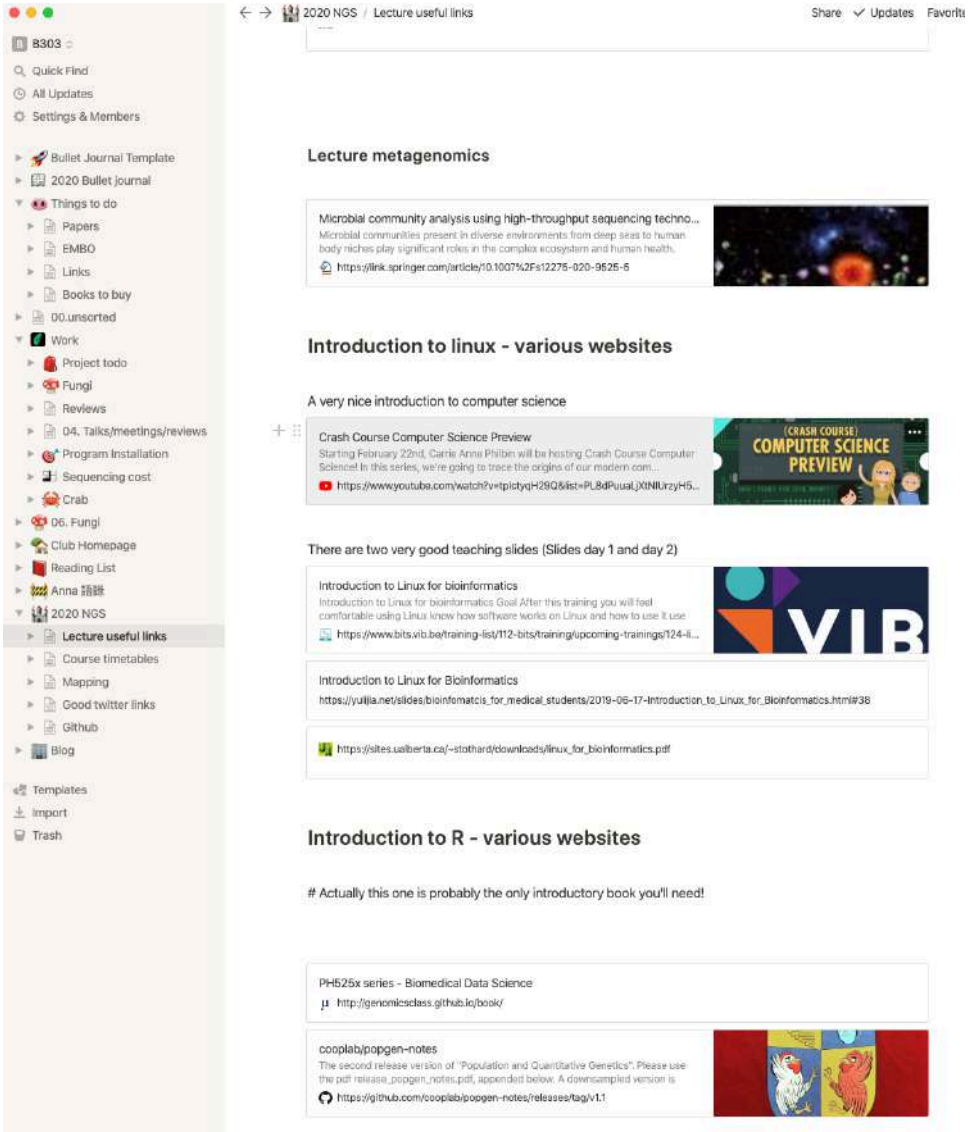
cd /mnt/nas2/ijt/nanopore/albacore/Fusarium-1D2-FAH14229

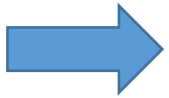
nohup read_fast5_basecaller.py -i /mnt/nas2/hmk/minion/20170928_1137_20170928-Fusarium-1D2/fast5/ -t 64 -s ./2.0.1.run1 -k SQK

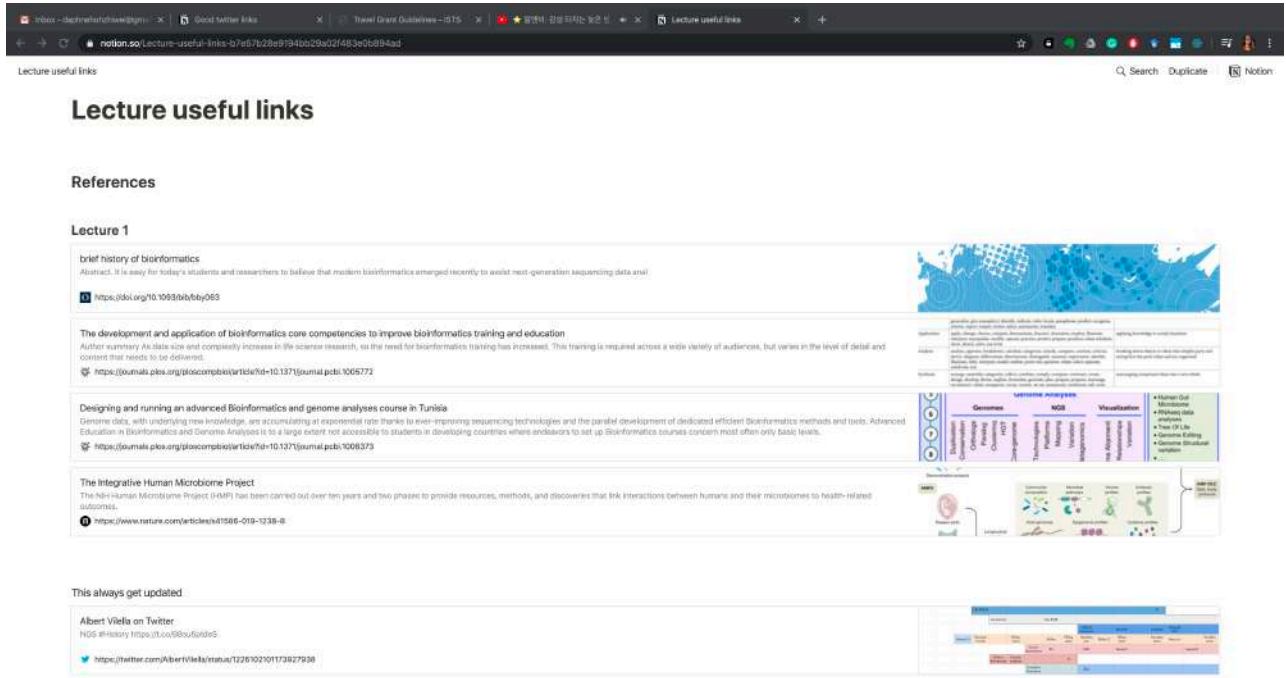
nohup [full_1dsq_basecaller.py](http://full_1dsq_basecaller.py/) -i /mnt/nas2/hmk/minion/20170928_1137_20170928-Fusarium-1D2/f

**# call again 2.2.7 1D 1D only**
```

Readily share / reproducible







Markdown and notebook ; Reproducible and redistributable

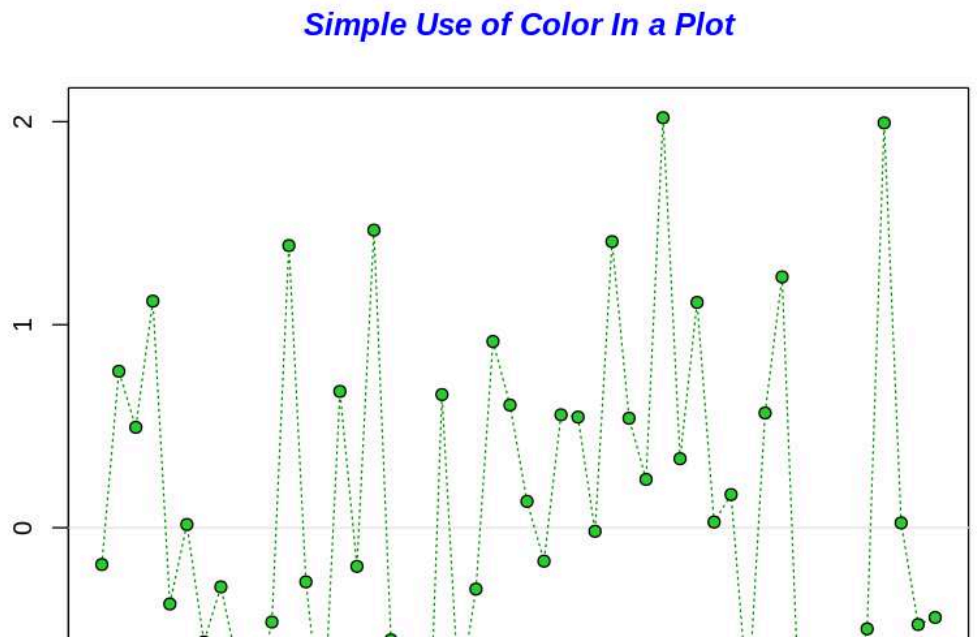
R demo

Here is some code which illustrates some of the differences between R and S graphics capabilities. Note that colors are generally specified by a character string name (taken from the X11 rgb.txt file) and that line textures are given similarly. The parameter "bg" sets the background parameter for the plot and there is also an "fg" parameter which sets the foreground color.

In [1]:
```
require(datasets)

require(grDevices); require(graphics)
```

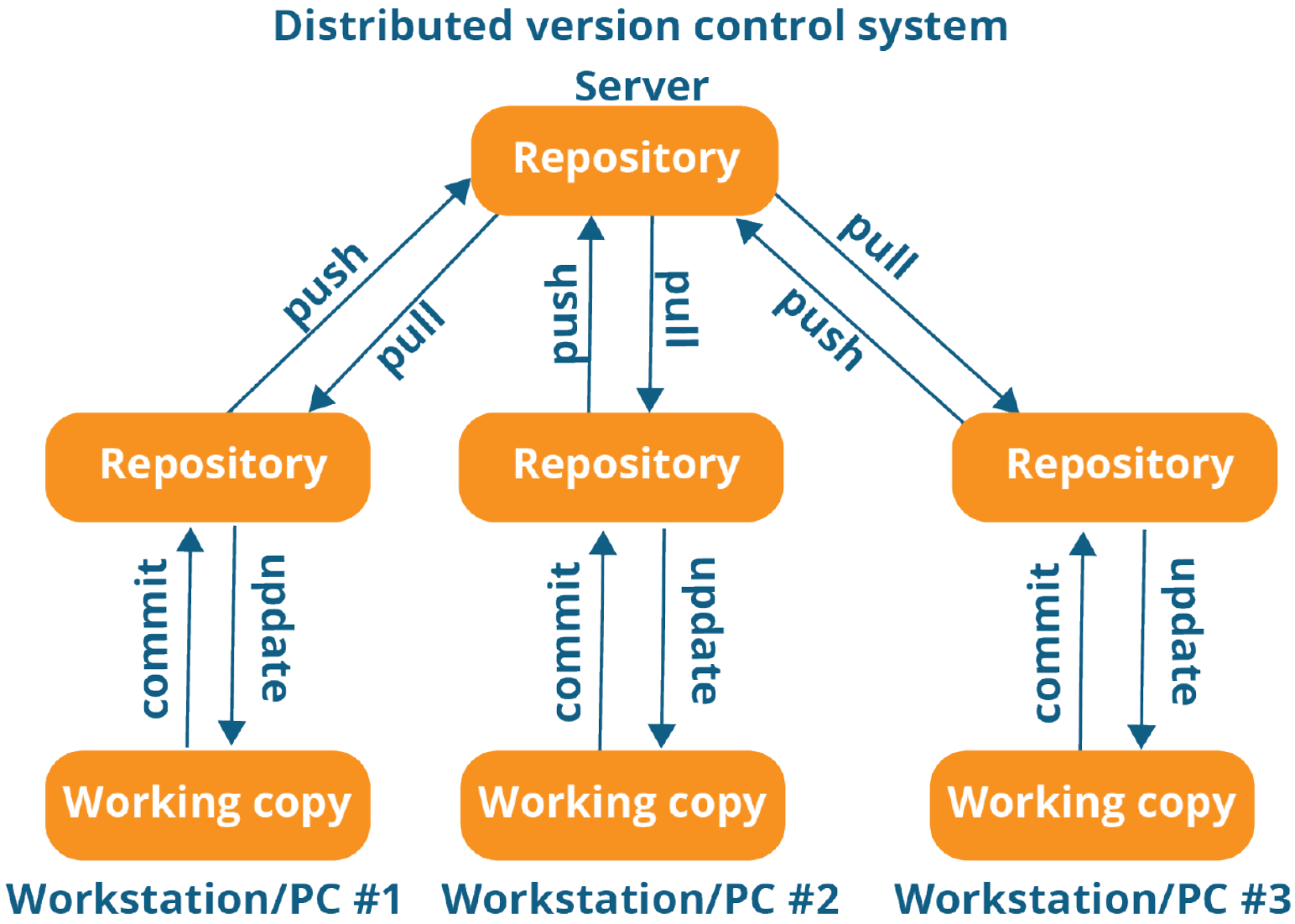
In [1]:
```
x <- stats::rnorm(50)
opar <- par(bg = "white")
plot(x, ann = FALSE, type = "n") +
abline(h = 0, col = gray(.90)) +
lines(x, col = "green4", lty = "dotted") +
points(x, bg = "limegreen", pch = 21) +
title(main = "Simple Use of Color In a Plot",
      xlab = "Just a Whisper of a Label",
      col.main = "blue", col.lab = gray(.8),
      cex.main = 1.2, cex.lab = 1.0, font.main = 4, font.lab = 3)
```

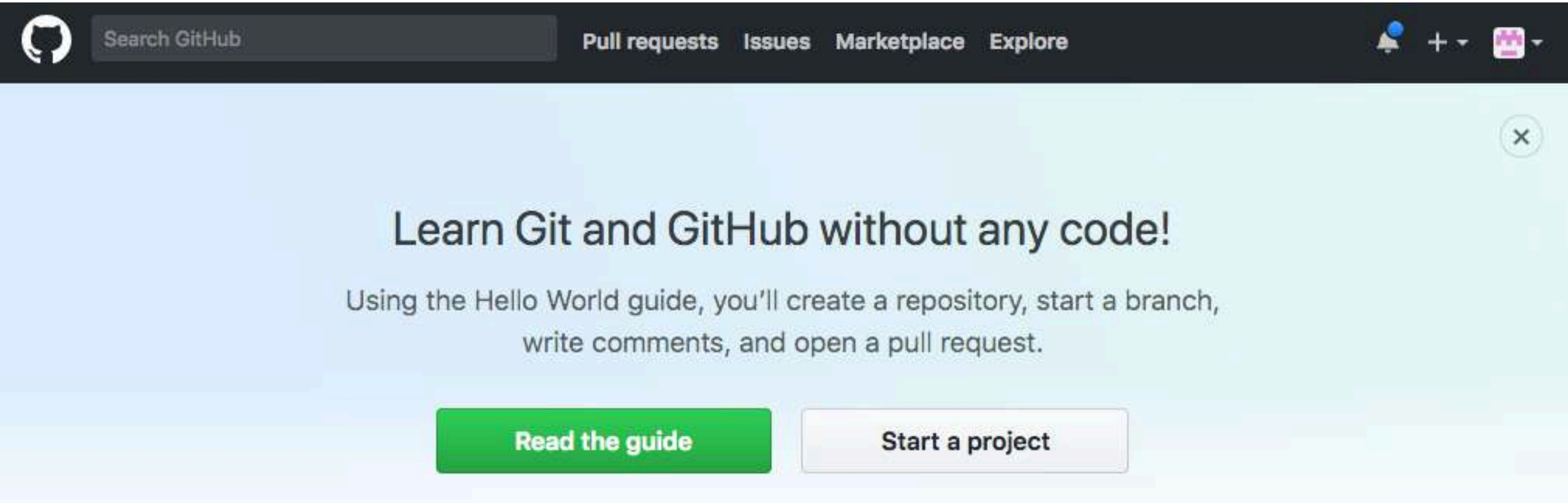


https://try.jupyter.org/

Version control: Git

git --fast-version-control





https://guides.github.com/activities/hello-world/

Use of markdown

- Created by John Gruber
- Informal plain-text formatting language
- Converts readable text to valid (X)HTML
- **Primary goal - readability**

Text using Markdown syntax	Text viewed in a browser

```
Heading
=======

## Sub-heading

Paragraphs are separated
by a blank line.

Two spaces at the end of a line
produces a line break.

Text attributes _italic_,
**bold**, `monospace`.

Horizontal rule:

---

Bullet list:

  * apples
  * oranges
  * pears

Numbered list:

  1. wash
  2. rinse
  3. repeat

A [link](http://example.com).

![Image](Image_icon.png)

> Markdown uses email-style > characters for blockquoting.

Inline <abbr title="Hypertext Markup Language">HTML</abbr>
is supported.
```

Heading

Sub-heading

Paragraphs are separated by a blank line.

Two spaces at the end of a line
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Text attributes *italic*, **bold**, `monospace`.

Horizontal rule:

Bullet list:

- apples
- oranges
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2. rinse
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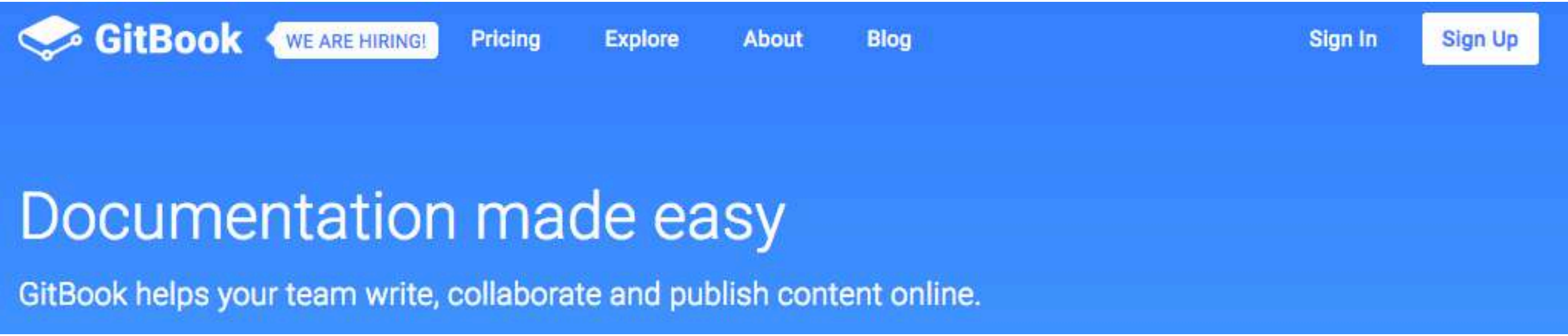
A link.

> Markdown uses email-style > characters for blockquoting.

Inline HTML is supported.

Git + Github + markdown



Some examples:

- https://cgsb.gitbooks.io/ngs-analysis/content/
- https://pfern.github.io/OSODOS/gitbook/

Lab communication (fb, LINE?; SLACK)

TOOLBOX

HOW SCIENTISTS USE SLACK

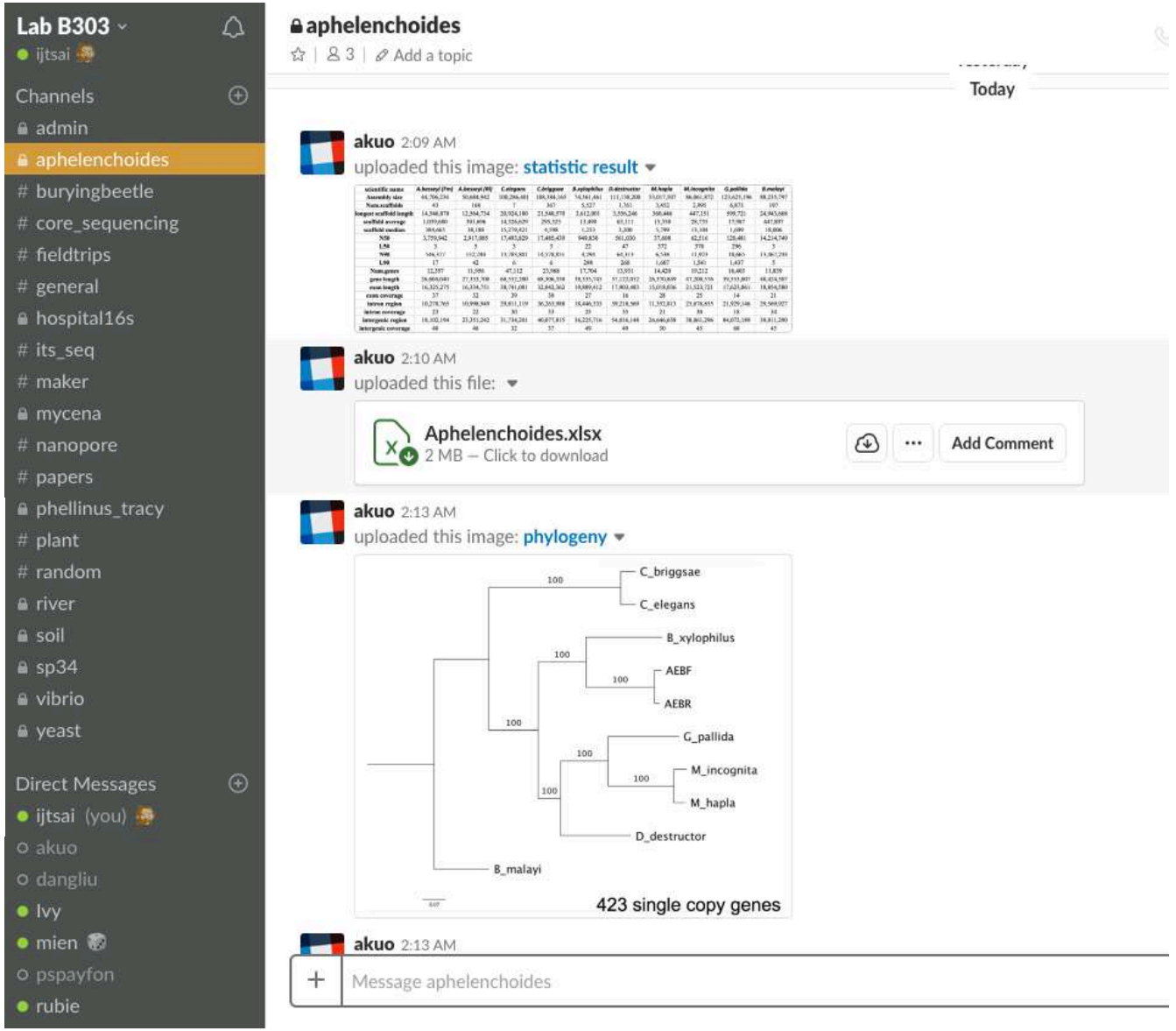
Eight ways labs benefit from the popular workplace messaging tool.

Amanda Leone 12:27 PM
Hi Anne we were planning on meeting 15 min before subgroup group meetings will you have time today?

anne_mcneil 1:00 PM
Yes, thanks for the reminder.

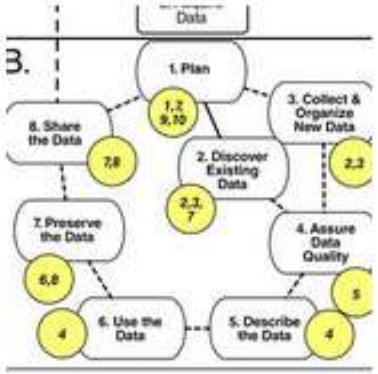
Amanda Leone 5:16 PM
preliminary result the DIBAL-H crude product looks good by NMR 🙌

anne_mcneil 5:20 PM
Woohoo



https://cen.acs.org/articles/94/i29/Slack-ing-helps-chemists-manage.html

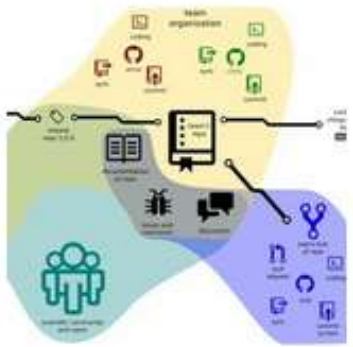
Ten simple rules series



Ten Simple Rules for Creating a Good Data Management Plan

William Michener

PLOS Computational Biology: 22 Oct 2015



Ten Simple Rules for Taking Advantage of Git and GitHub

Yasset Perez-Riverol, Laurent Gatto, Rui Wang, Timo Sachsenberg, Julian Uszkoreit, Felipe da Veiga Leprevost, …

PLOS Computational Biology: 14 Jul 2016

Ten Simple Rules for a Computationa Biologist's Laboratory Notebook

Santiago Schnell

PLOS Computational Biology: 10 Sep 2015

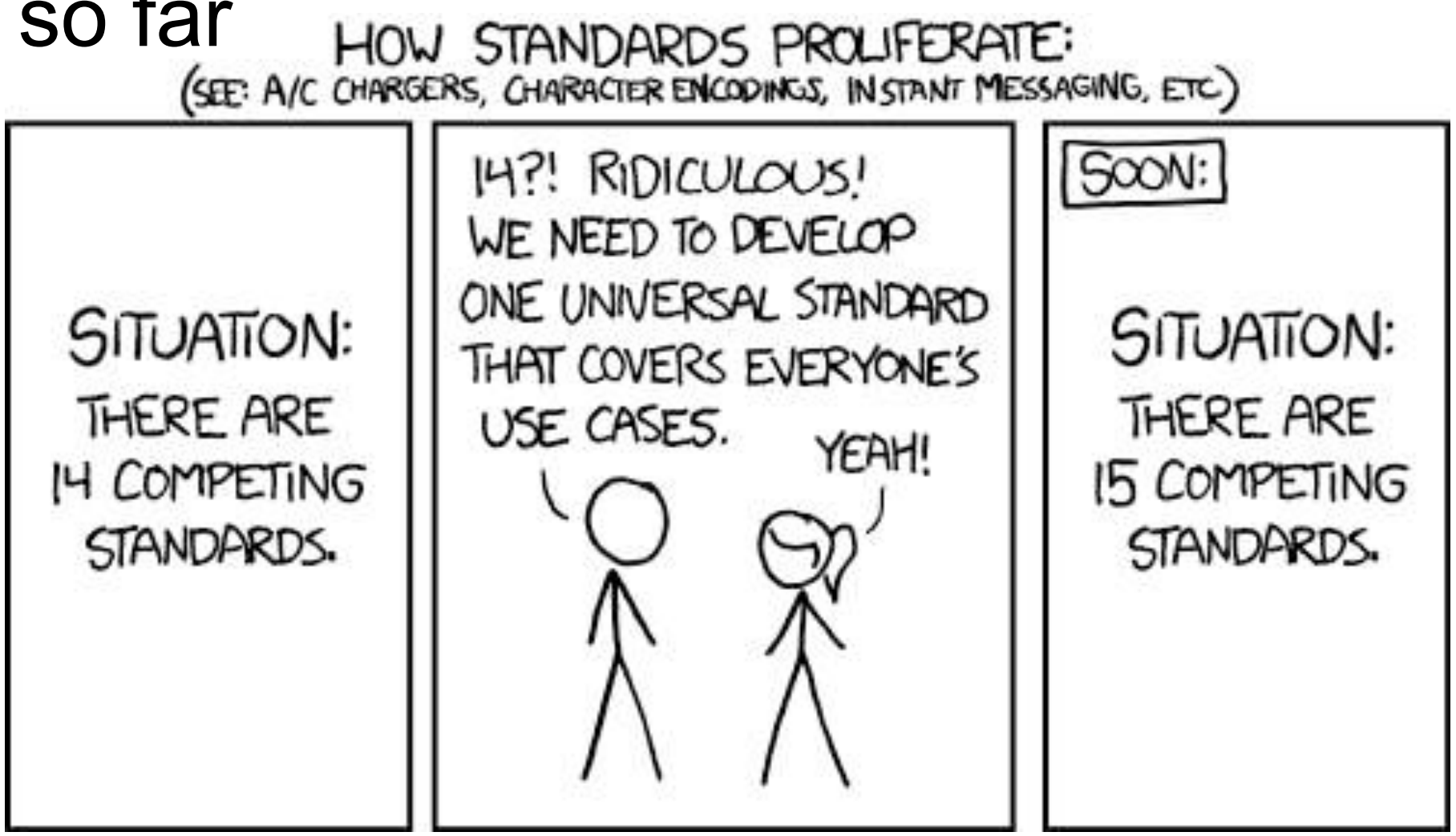
Ten simple rules for biologists learning to program

Maureen A. Carey, Jason A. Papin

PLOS Computational Biology: 04 Jan 2018

http://collections.plos.org/ten-simple-rules

Summary so far



https://xkcd.com/927/

- No need to do everything ‘perfect’
- Depending on scale, use something that is most effective

Useful links:

A series of Jypyter notebooks hosted on github

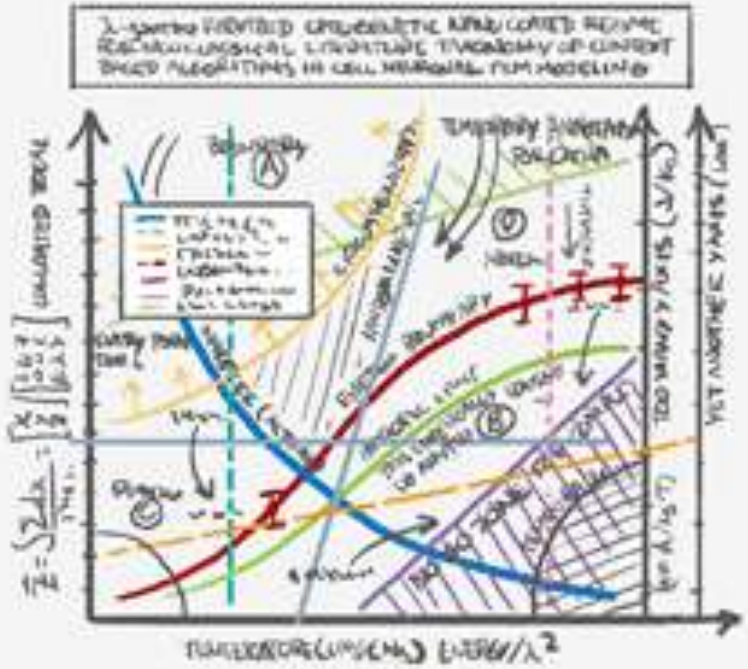
- https://github.com/jupyter/jupyter/wiki/A-gallery-of-interesting-Jupyter-Notebooks

Other links

- http://linux.vbird.org/linux_basic/ (Chinese ; extremely useful) ****
- https://evomics.org/learning/unix-tutorial/
- http://www.ark-genomics.org/events-online-training-eu-training-course/introduction-linux
- http://linuxcommand.org/

Data type / Visualisations

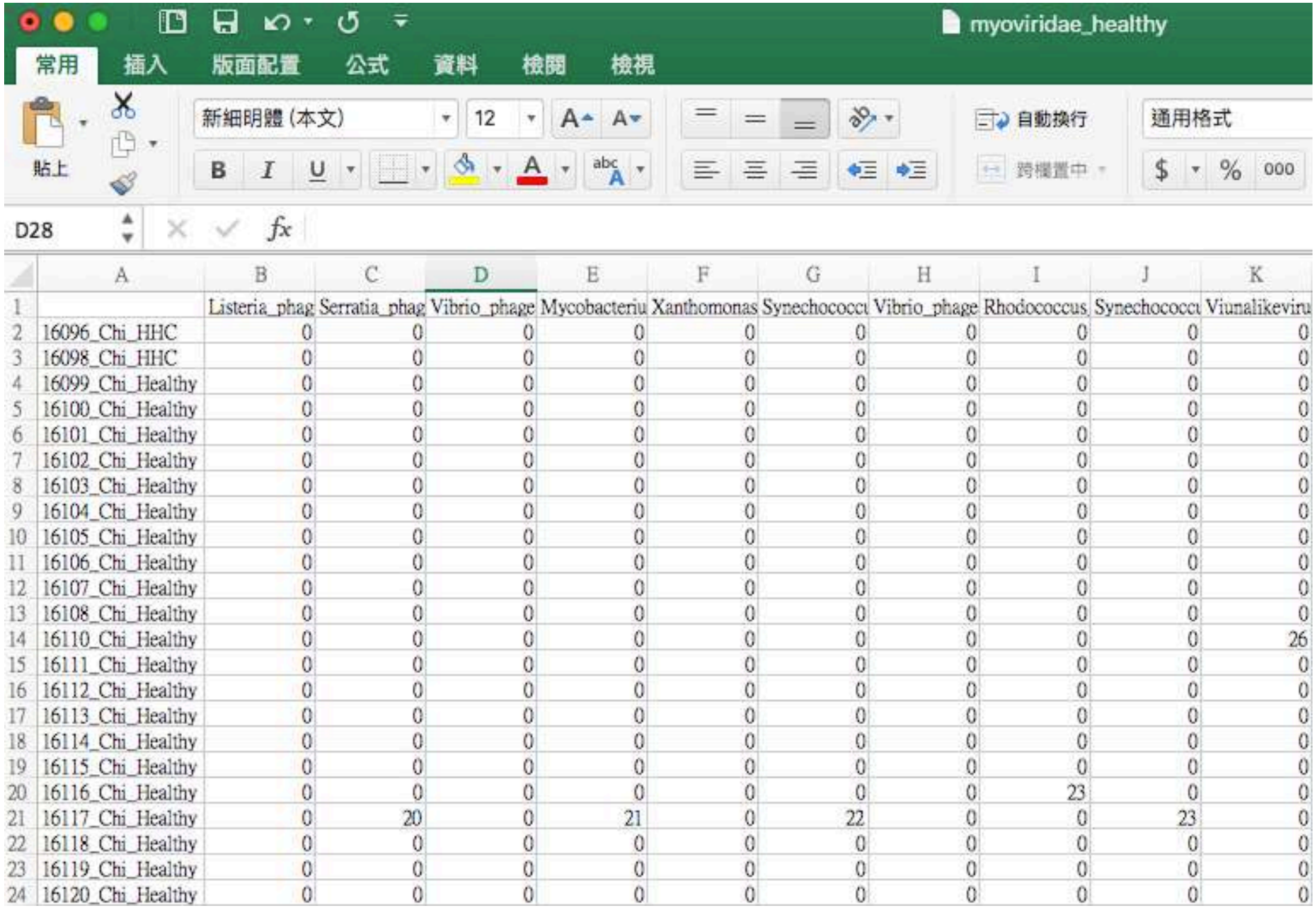
A PICTURE IS WORTH A THOUSAND WORDS.



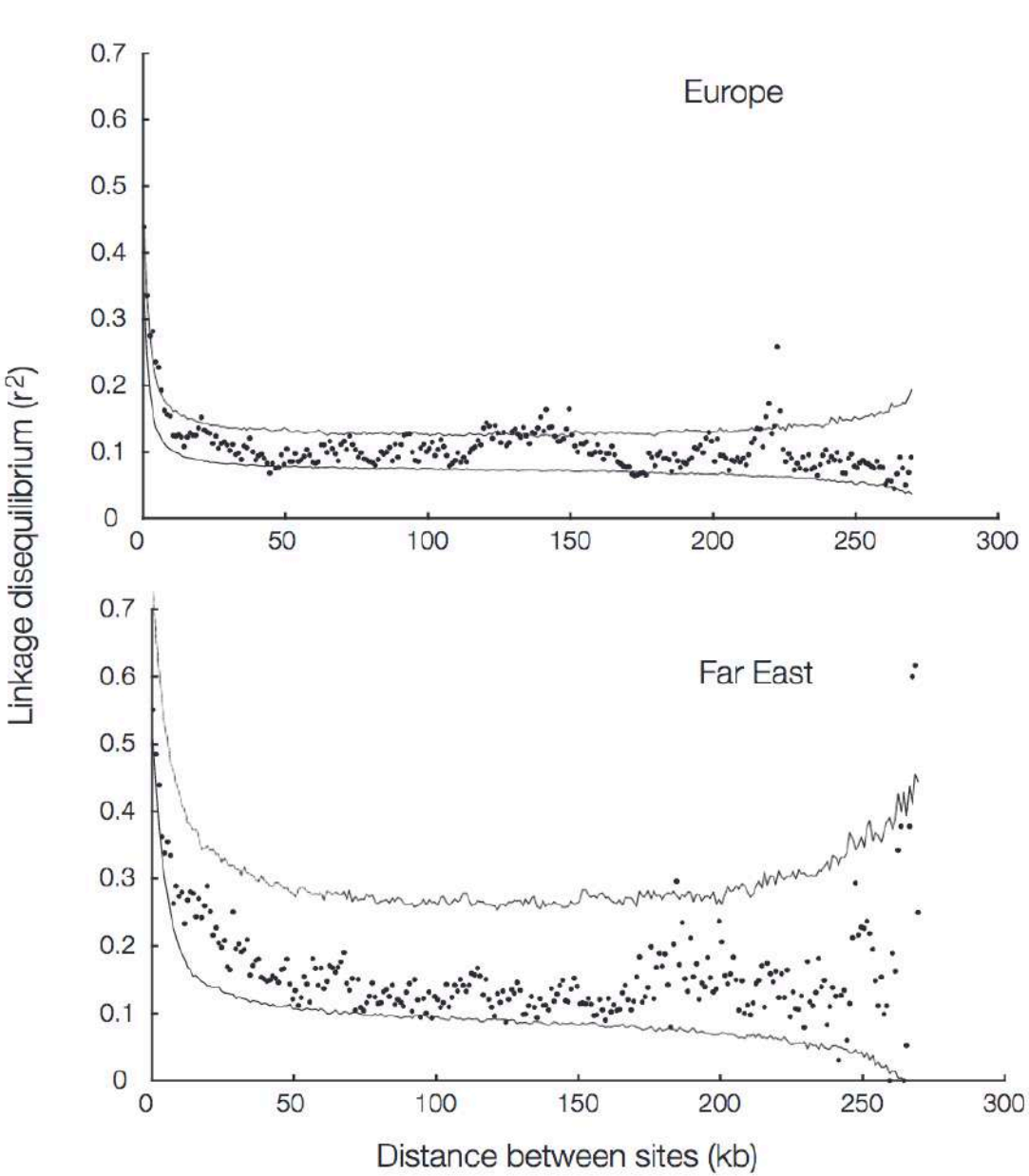
WOW!

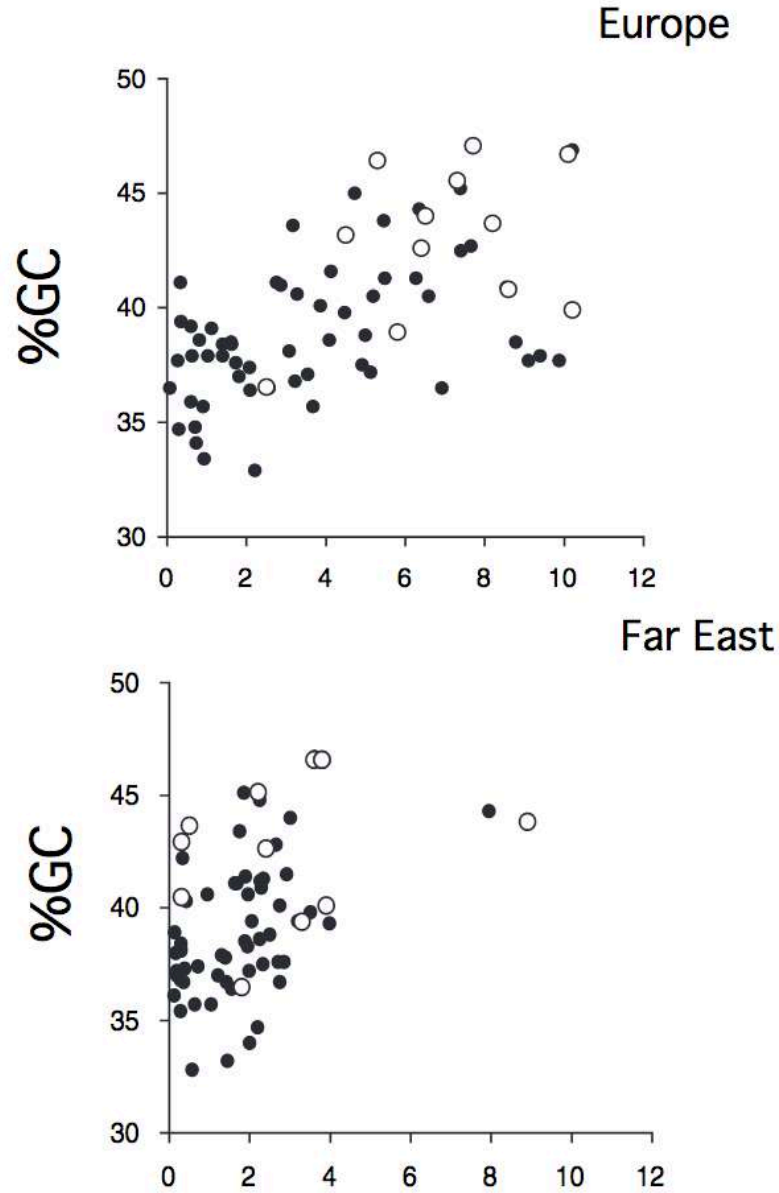
FIGURE 1.

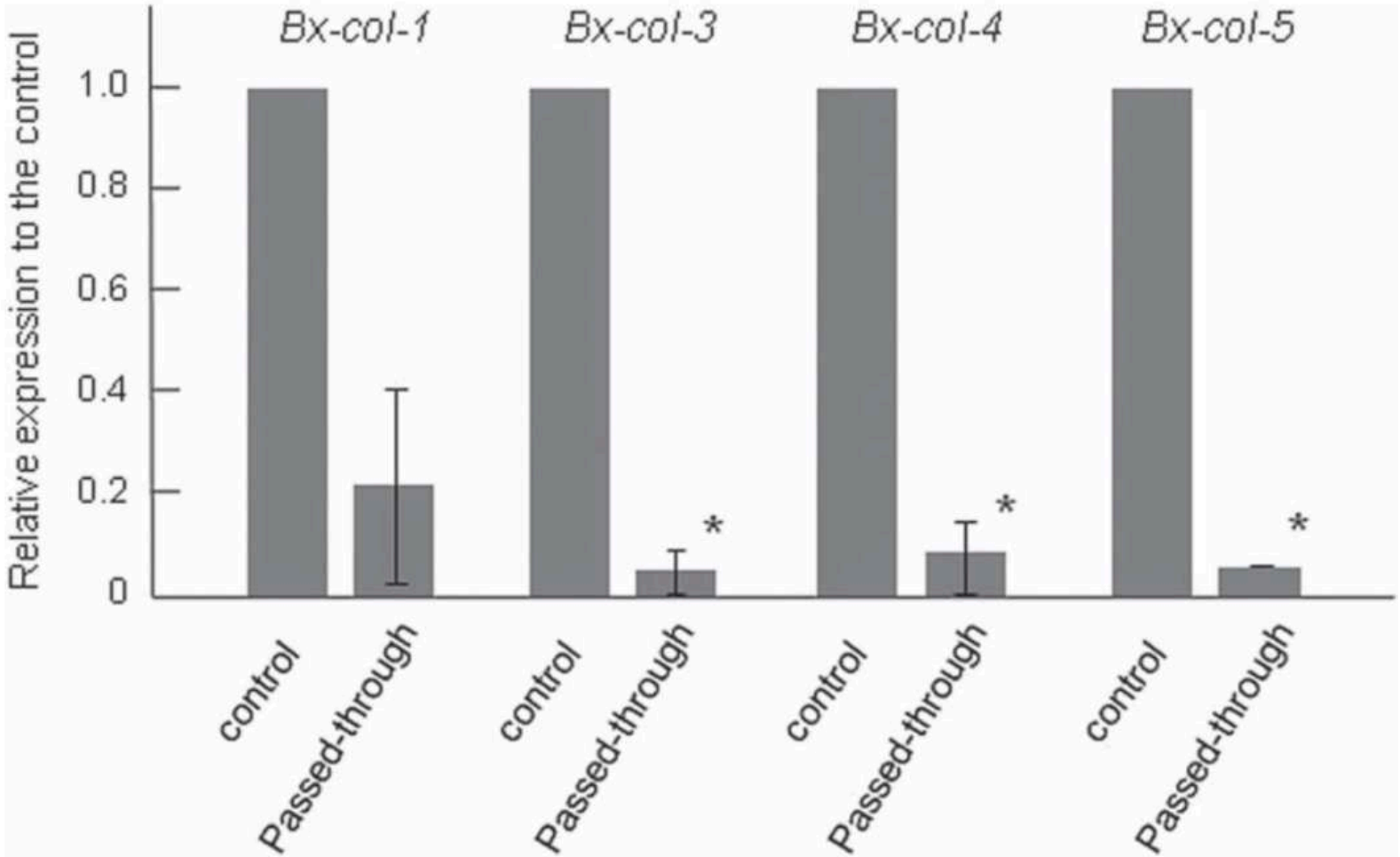
A PICTURE WITH A THOUSAND WORDS IS USUALLY WORTH A PH.D.

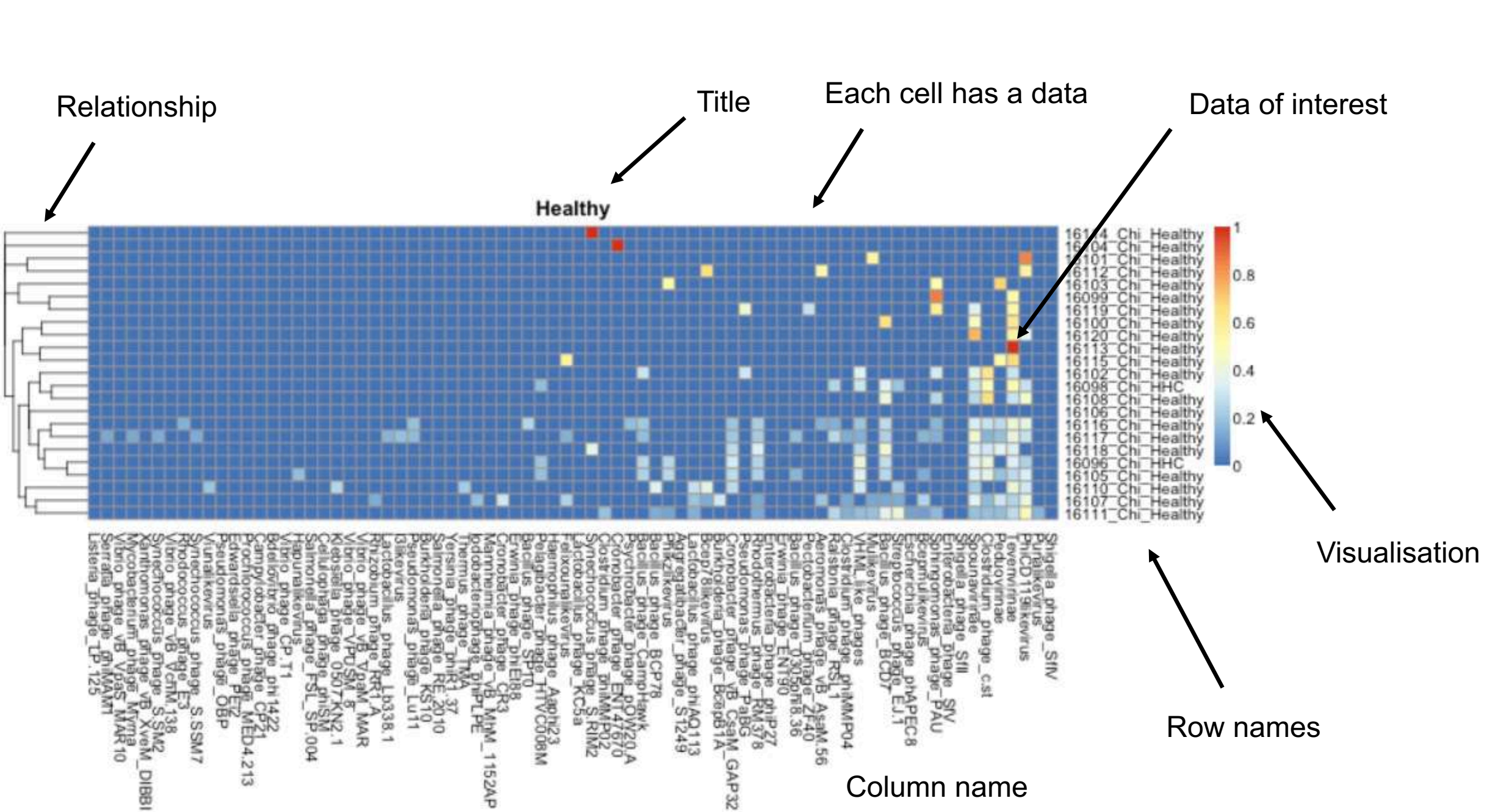


	A	B	C	D	E	F	G	H	I	J	K
1		Listeria_phag	Serratia_phag	Vibrio_phage	Mycobacteriu	Xanthomonas	Synechococci	Vibrio_phage	Rhodococcus_	Synechococci	Viunalikeviru
2	16096_Chi_HHC	0	0	0	0	0	0	0	0	0	0
3	16098_Chi_HHC	0	0	0	0	0	0	0	0	0	0
4	16099_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
5	16100_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
6	16101_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
7	16102_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
8	16103_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
9	16104_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
10	16105_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
11	16106_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
12	16107_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
13	16108_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
14	16110_Chi_Healthy	0	0	0	0	0	0	0	0	0	26
15	16111_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
16	16112_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
17	16113_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
18	16114_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
19	16115_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
20	16116_Chi_Healthy	0	0	0	0	0	0	0	23	0	0
21	16117_Chi_Healthy	0	20	0	21	0	22	0	0	23	0
22	16118_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
23	16119_Chi_Healthy	0	0	0	0	0	0	0	0	0	0
24	16120_Chi_Healthy	0	0	0	0	0	0	0	0	0	0


Europe
Far East
Linkage disequilibrium (r^2)
Distance between sites (kb)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
300

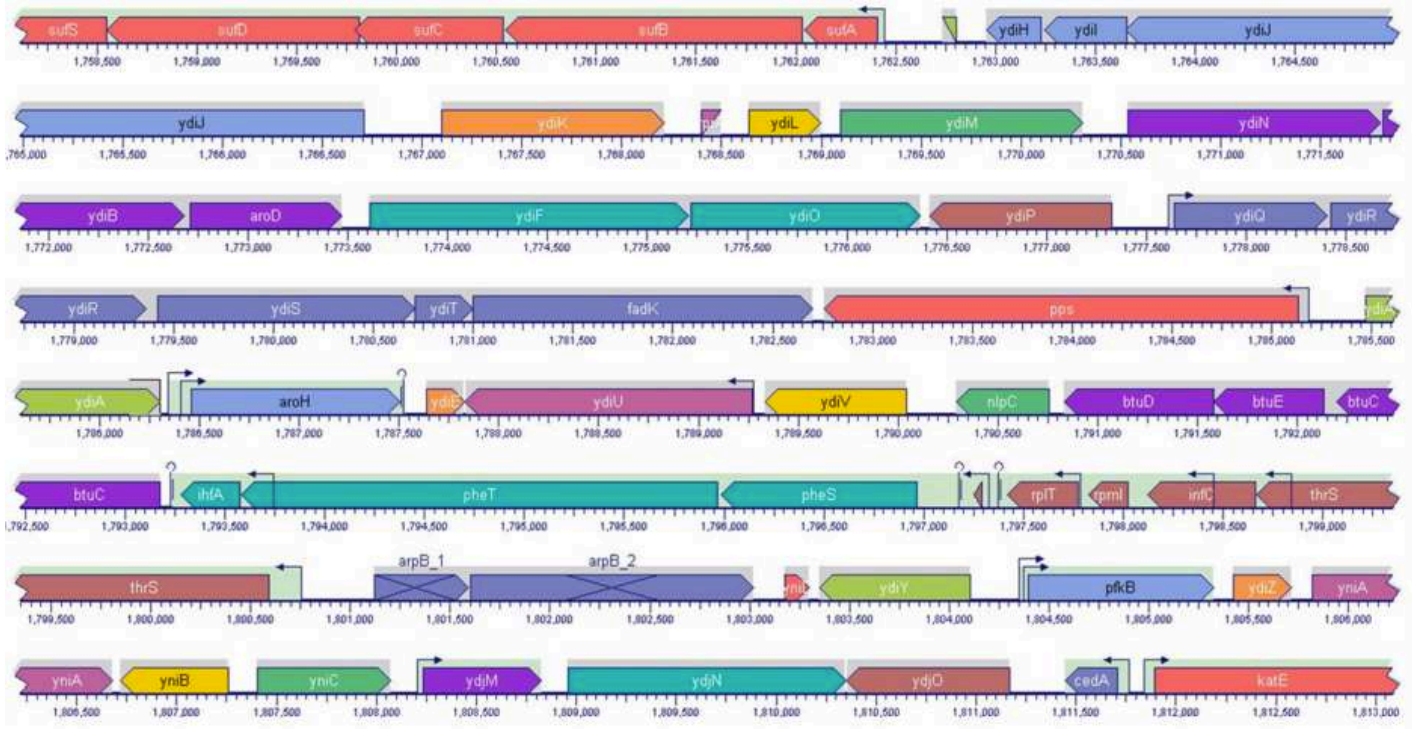

Europe
Far East
%GC
50
45
40
35
30
0
2
4
6
8
10
12


Bx-col-1
Bx-col-3
Bx-col-4
Bx-col-5
Relative expression to the control
1.0
0.8
0.6
0.4
0.2
0
*
*
*
control
Passed-through
control
Passed-through
control
Passed-through
control
Passed-through

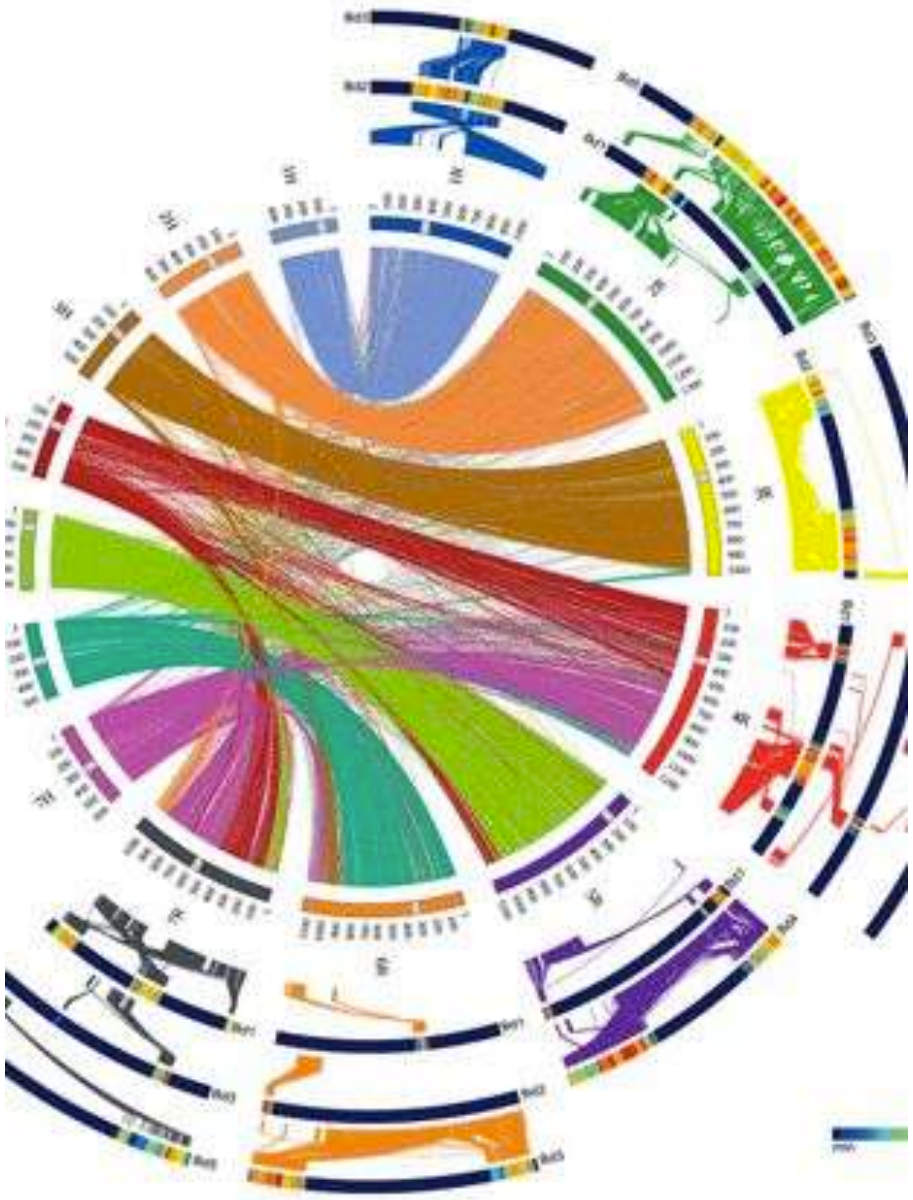

Relationship
Title
Each cell has a data
Data of interest
Healthy
1
0.8
0.6
0.4
0.2
0
Visualisation
Row names
Column name

Locations / maps

- How do we represent/visualise them?

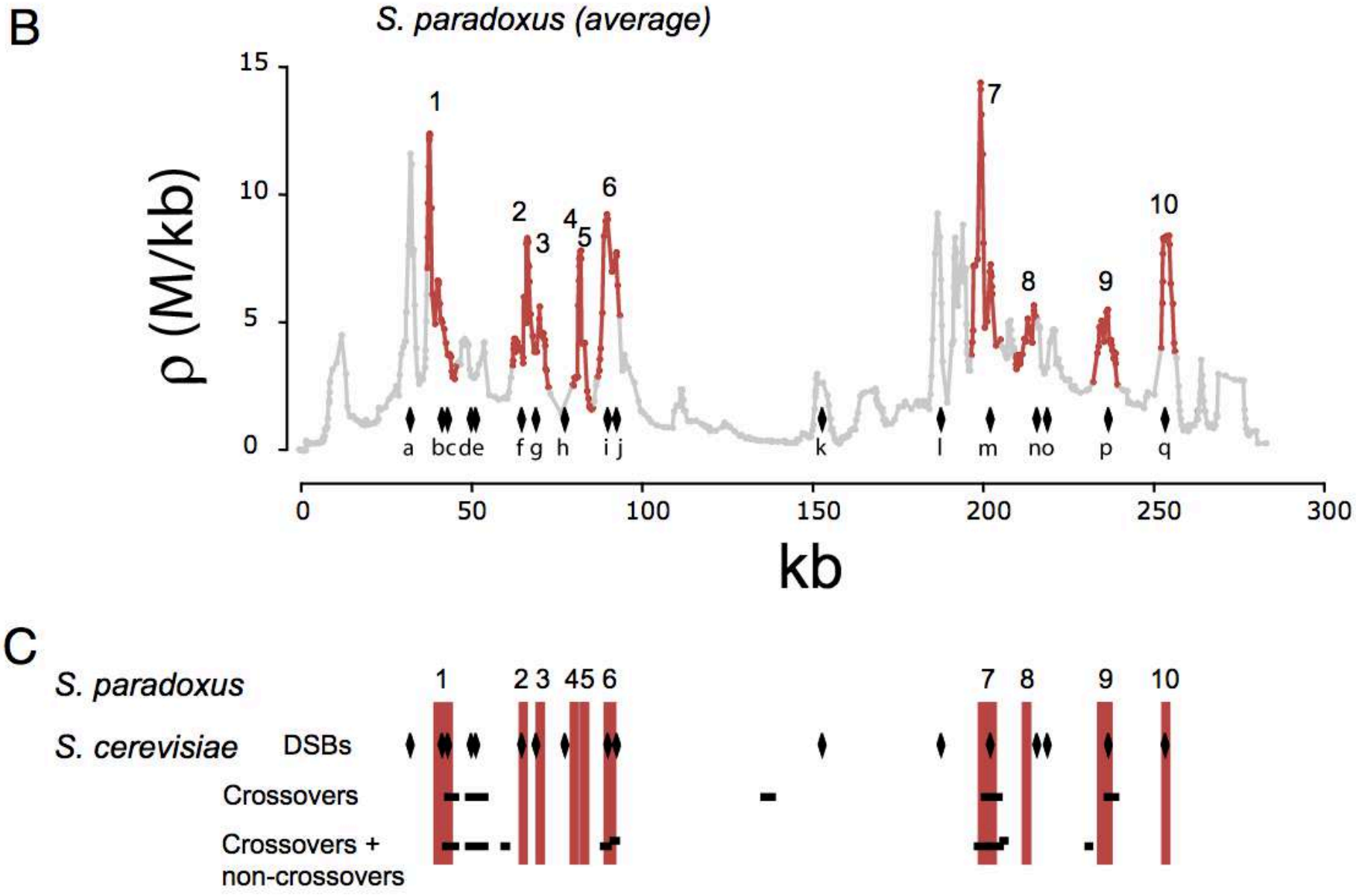


Gene locations / strand

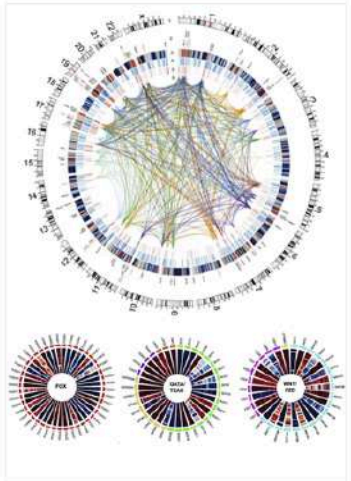


Circos

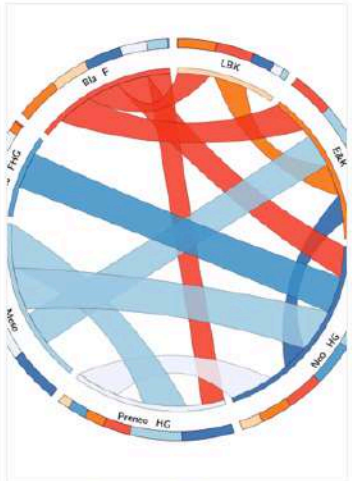
Properties on the genome



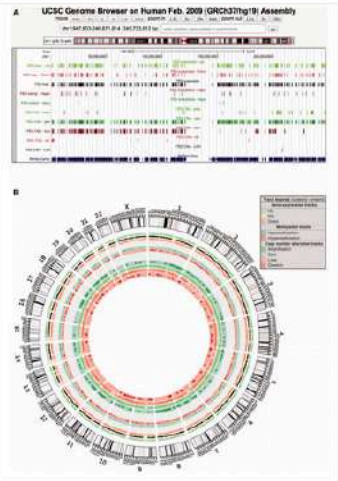
Visualising genomes - Circos



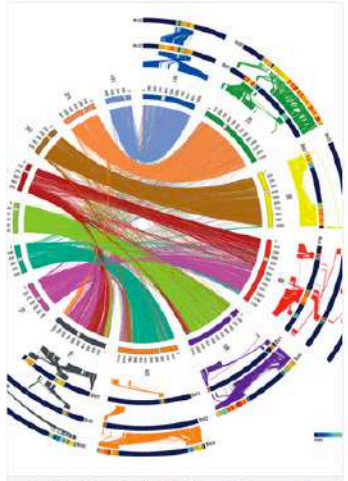
▲ 1 - 1 Dec 2013 | Saben J, Zhong Y, McKelvey S *et al.* (2014) A comprehensive analysis of the human placenta transcriptome *Placenta* **35**:125-131.



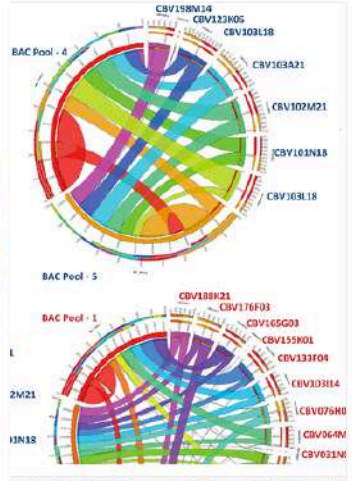
▲ 2 - 25 Oct 2013 | Bollongino R, Nehlich O, Richards MP *et al.* (2013) 2000 years of parallel societies in Stone Age Central Europe *Science* **342**:479-481.



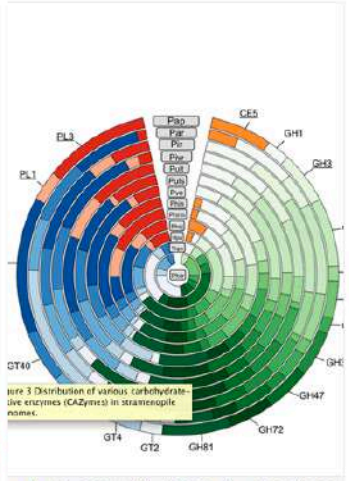
▲ 3 - 25 Oct 2013 | Dayem Ullah AZ, Cutts RJ, Ghetia M *et al.* (2013) The pancreatic expression database: recent extensions and updates *Nucleic Acids Res*



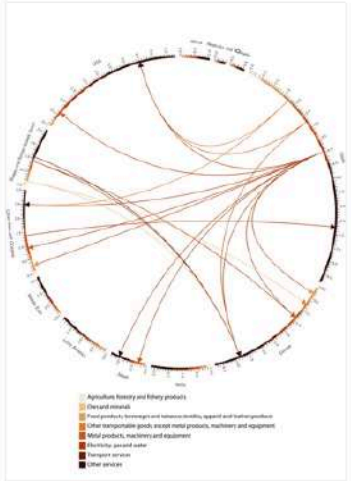
▲ 13 - 8 Oct 2013 | Martis MM, Zhou R, Haseneyer G *et al.* (2013) Reticulate Evolution of the Rye Genome *Plant Cell*



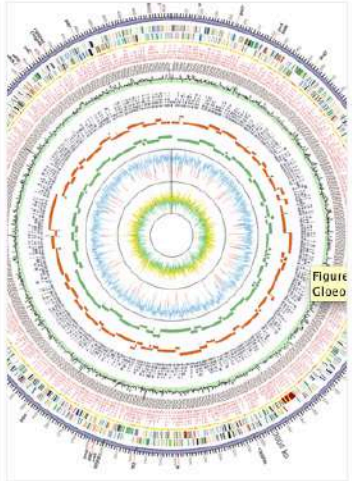
▲ 14 - 8 Oct 2013 | Buyyarapu R, Kantety RV, Yu JZ *et al.* (2013) BAC-Pool Sequencing and Analysis of Large Segments of A12 and D12 Homoeologous Chromosomes in Upland Cotton *PLoS One* **8**:e76757.



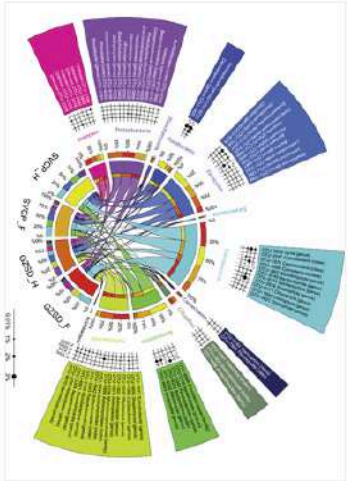
▲ 15 - 4 Oct 2013 | Adhikari BN, Hamilton JP, Zerillo MM *et al.* (2013) Comparative Genomics Reveals Insight into Virulence Strategies of Plant Pathogenic Oomycetes *PLoS One* **8**:e75072.



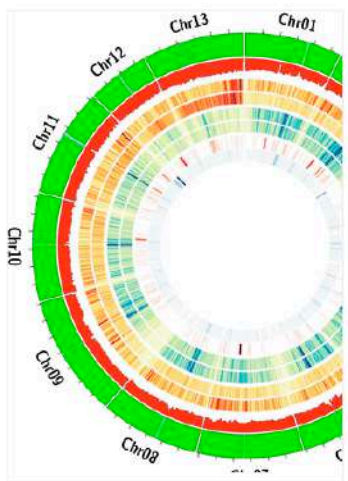
▲ 4 - 23 Oct 2013 | Kanemoto K, Moran D, Lenzen M *et al.* (2013) International trade undermines national emission reduction targets: New evidence from air pollution *Global Environmental Change*



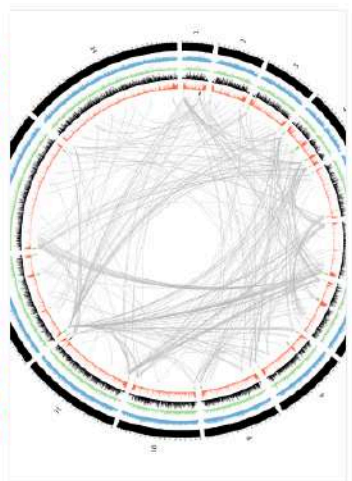
▲ 5 - 23 October 2013 | Saw JHW, Schatz M, Brown MV *et al.* (2013) Cultivation and Complete Genome Sequencing of Gloeobacter kilaueensis sp. nov., from a Lava Cave in Kilauea Caldera, Hawai'i *PLoS One* **8**:e76376.



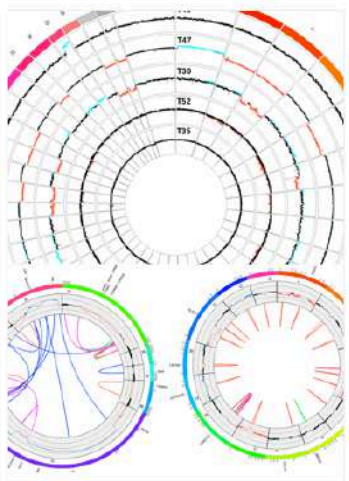
▲ 6 - 17 Oct 2013 | Ye L, Amberg J, Chapman D *et al.* (2013) Fish gut microbiota analysis differentiates physiology and behavior of invasive Asian carp and indigenous American fish *The ISME Journal*



▲ 16 - 1 Oct 2013 | Page JT, Huynh MD, Liechty ZS *et al.* (2013) Insights into the Evolution of Cotton Diploids and Polyploids from Whole-Genome Re-sequencing *G3: Genes|Genomes|Genetics* **3**:1809-1818.



▲ 17 - 30 Sep 2013 | Lemieux JE, Kyes SA, Otto TD *et al.* (2013) Genome-wide profiling of chromosome interactions in Plasmodium falciparum characterizes nuclear architecture and reconfigurations associated with antigenic variation *Molecular microbiology*



▲ 18 - 30 Sep 2013 | Beck J, Hennecke S, Bornemann-Kolatzki K *et al.* (2013) Genome Aberrations in Canine Mammary Carcinomas and Their Detection in Cell-Free Plasma DNA *PLoS One* **8**:e75485.

BED/gff format

- Features on genome use bed / gff files to represent their locations
- “Optional field” can be added for additional information

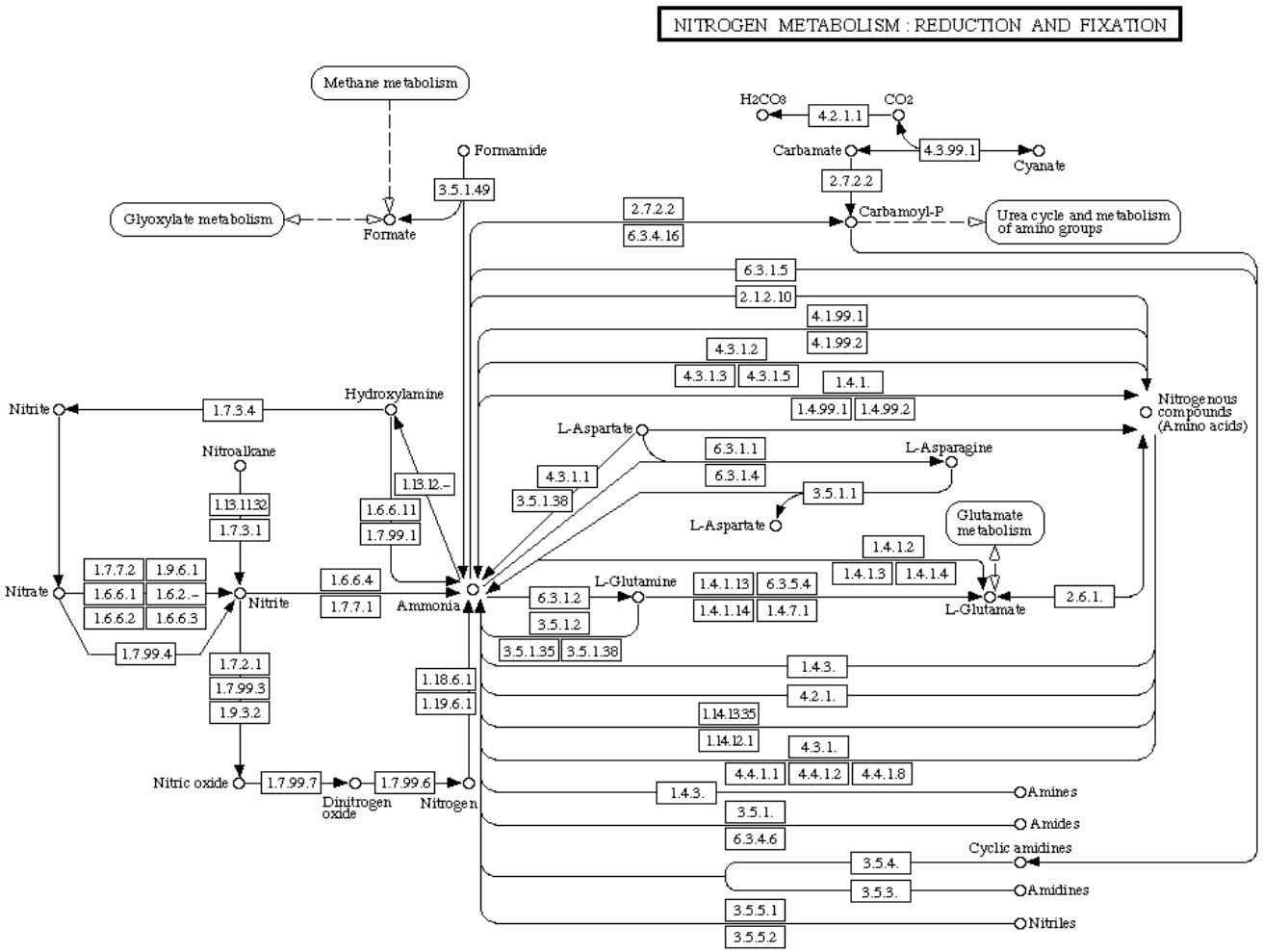
```
chr7    127471196  127472363
chr7    127472363  127473530
chr7    127473530  127474697
chr7    127474697  127475864
chr7    127475864  127477031
chr7    127477031  127478198
chr7    127478198  127479365
chr7    127479365  127480532
chr7    127480532  127481699
```

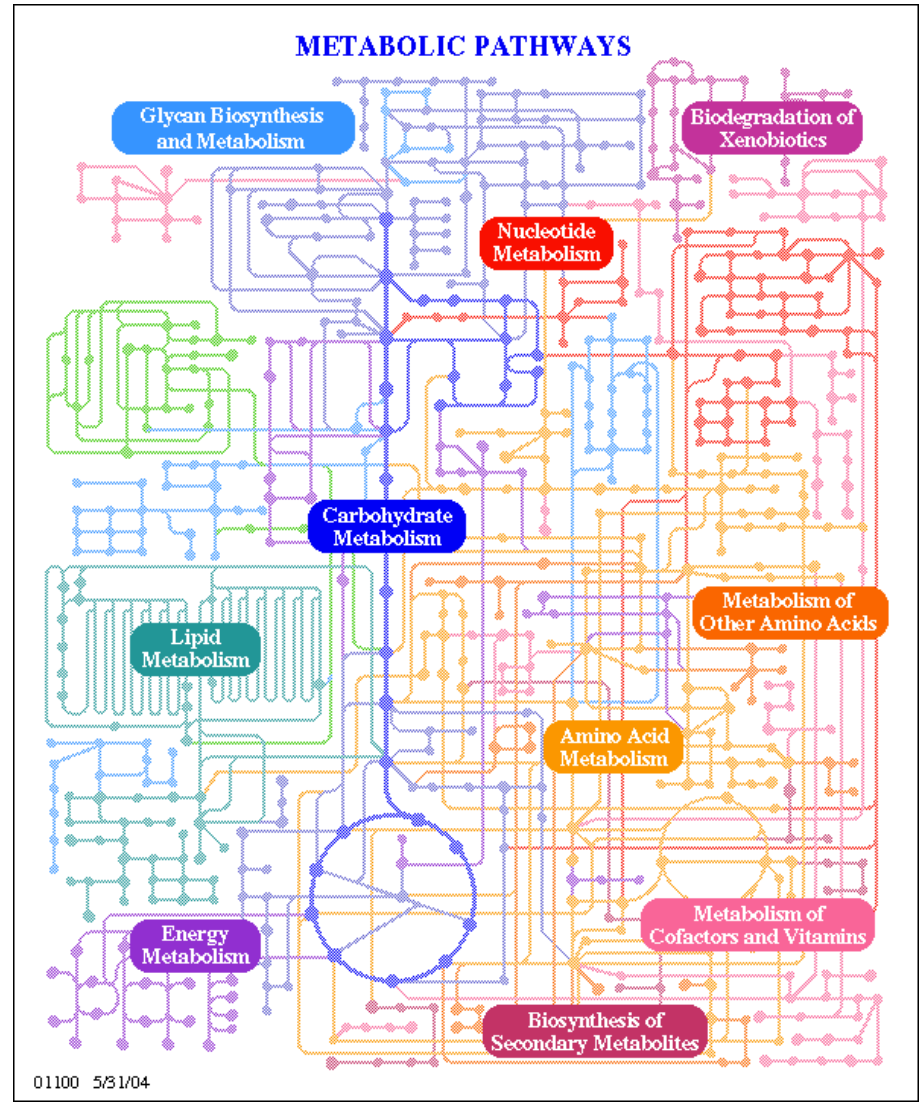
```
IV     curated exon    5506900 5506996 . + .   Transcript B0273.1
IV     curated exon    5506026 5506382 . + .   Transcript B0273.1
IV     curated exon    5506558 5506660 . + .   Transcript B0273.1
IV     curated exon    5506738 5506852 . + .   Transcript B0273.1
```

http://genome.ucsc.edu/FAQ/FAQformat#format1

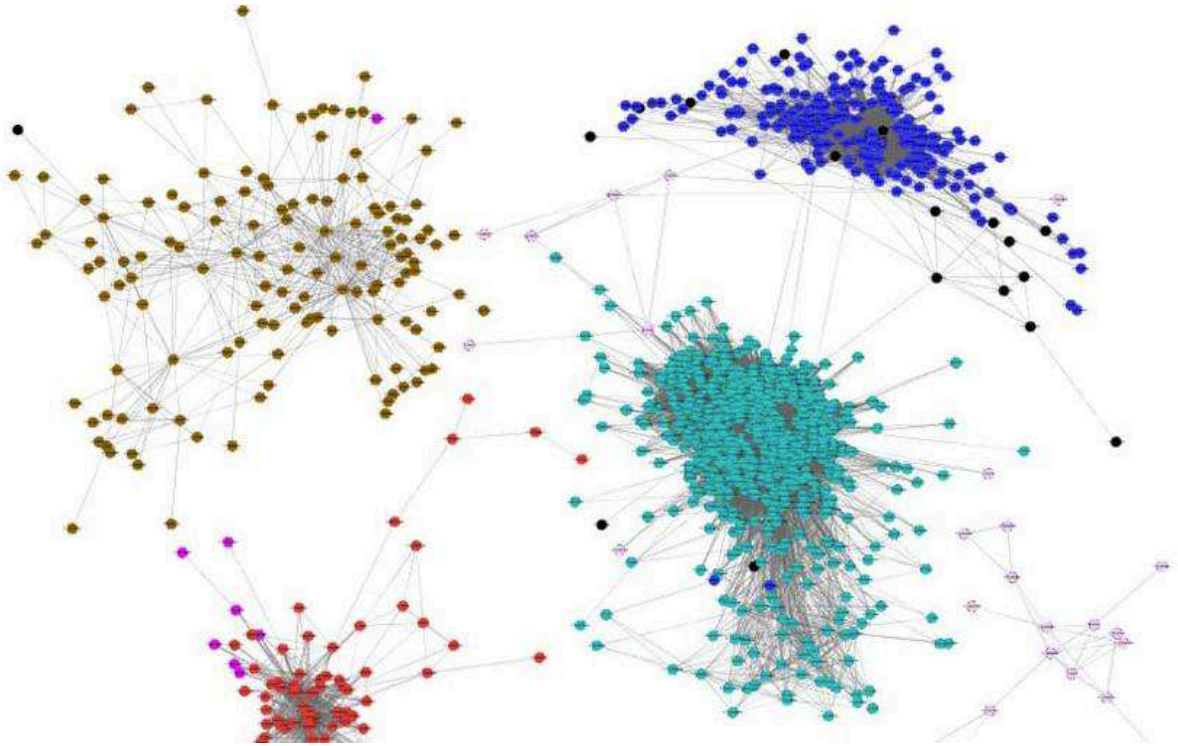
http://gmod.org/wiki/GFF2

Pathways


NITROGEN METABOLISM : REDUCTION AND FIXATION
Methane metabolism
Formamide
Glyoxylate metabolism
Formate
H2CO3
CO2
Carbamate
Cyanate
Carbamoyl-P
Urea cycle and metabolism of amino groups
Hydroxylamine
Nitrite
Nitroalkane
Nitrate
L-Aspartate
L-Asparagine
Glutamate metabolism
L-Glutamine
L-Glutamate
Ammonia
Nitrogenous compounds (Amino acids)
Nitric oxide
Dinitrogen oxide
Nitrogen
Amines
Amides
Cyclic amidines
Amidines
Nitriles


METABOLIC PATHWAYS
Glycan Biosynthesis and Metabolism
Biodegradation of Xenobiotics
Nucleotide Metabolism
Carbohydrate Metabolism
Lipid Metabolism
Metabolism of Other Amino Acids
Amino Acid Metabolism
Energy Metabolism
Metabolism of Cofactors and Vitamins
Biosynthesis of Secondary Metabolites
01100 5/31/04

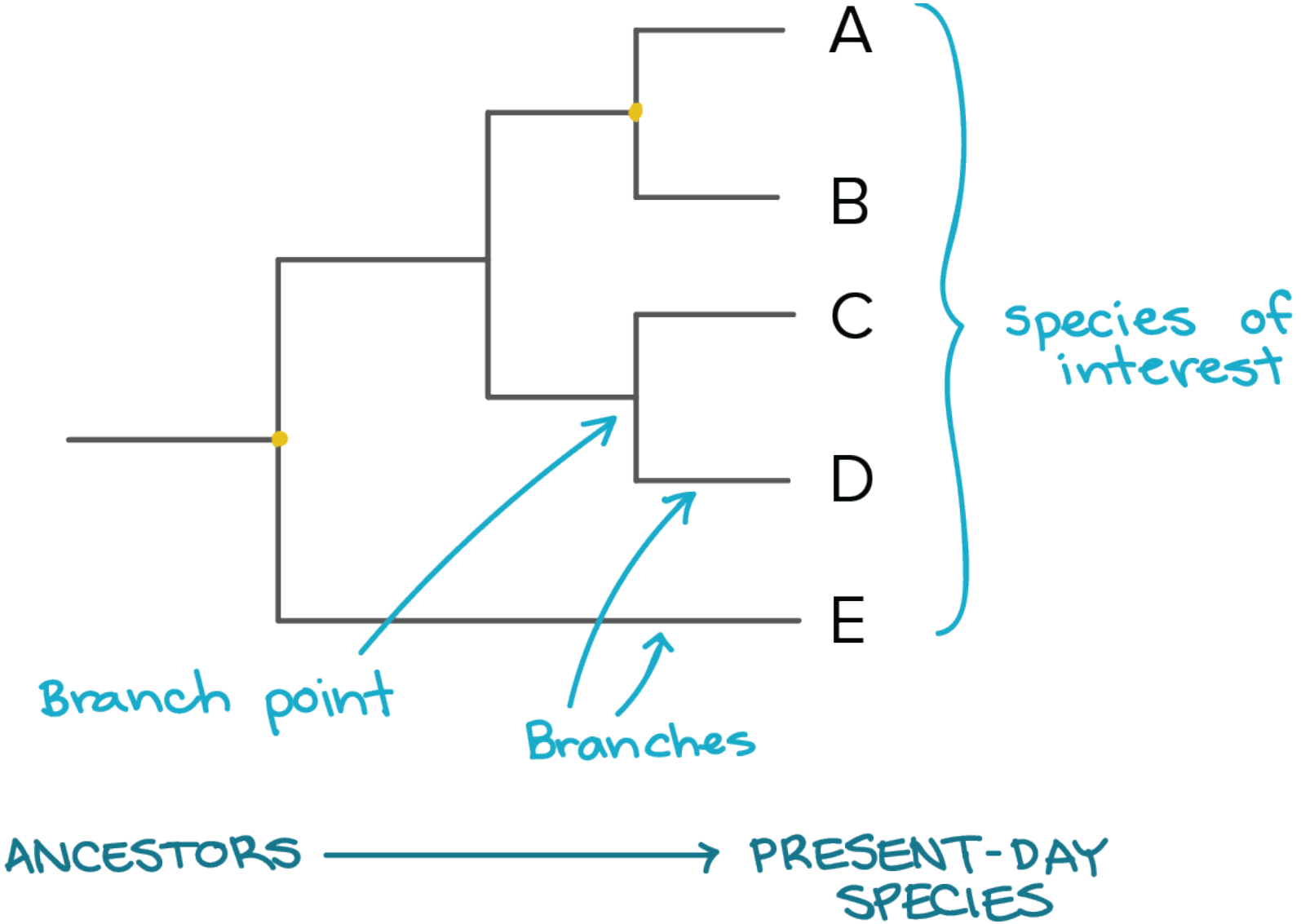
Importance of networks in biology



Gene interaction networks

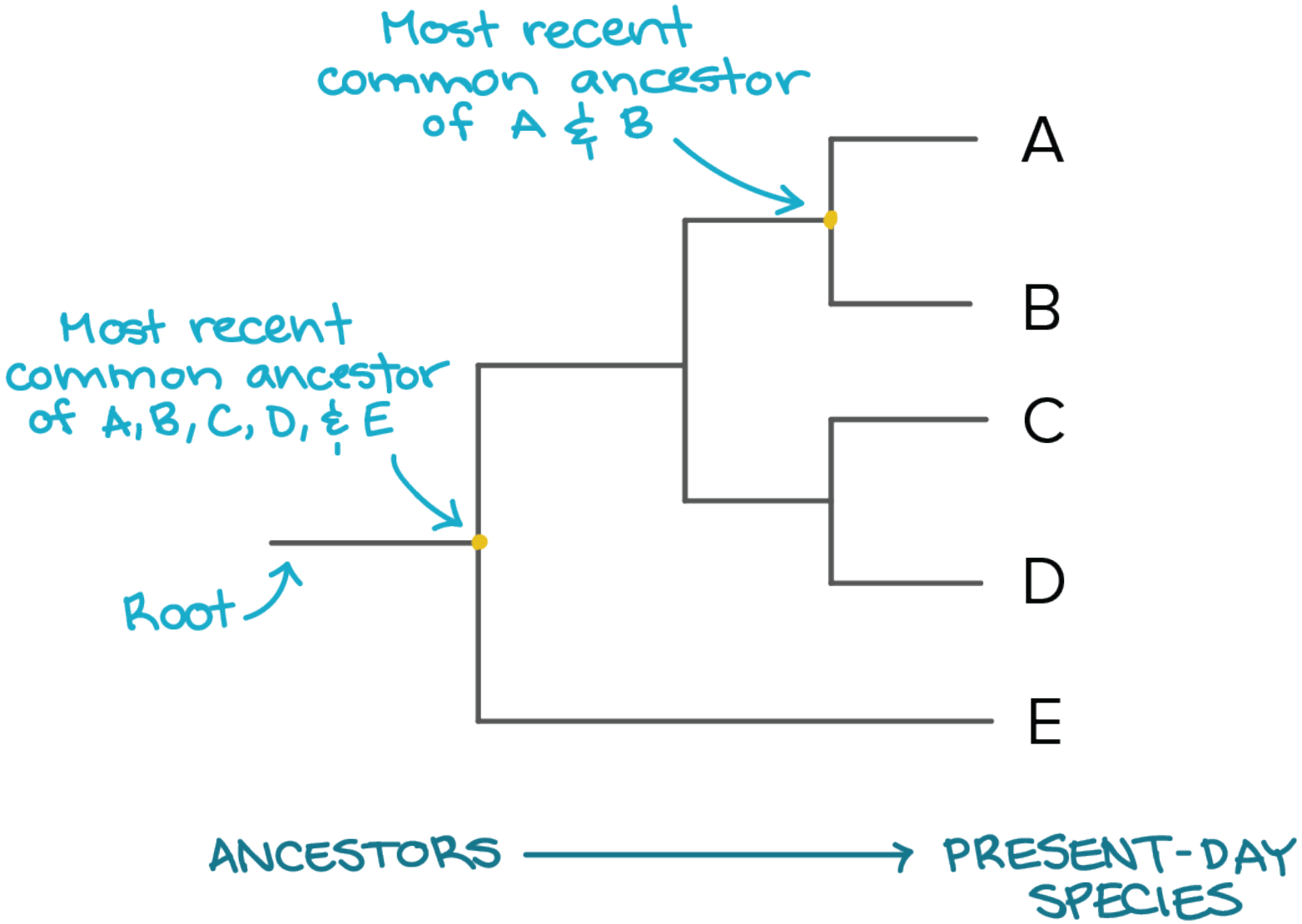
Protein interaction network

Phylogeny



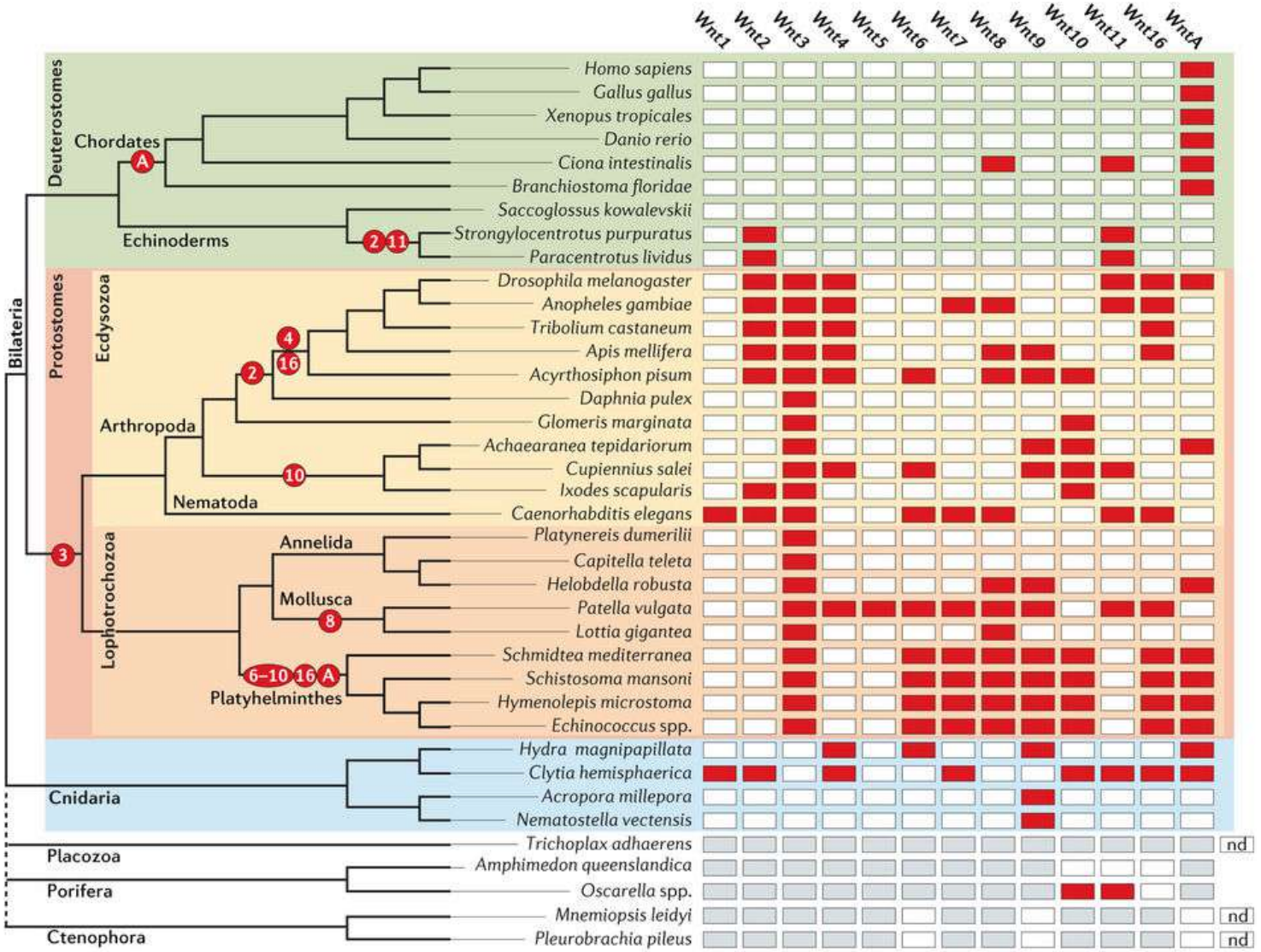
https://www.khanacademy.org/science/biology/her/tree-of-life/a/phylogenetic-trees

Phylogeny



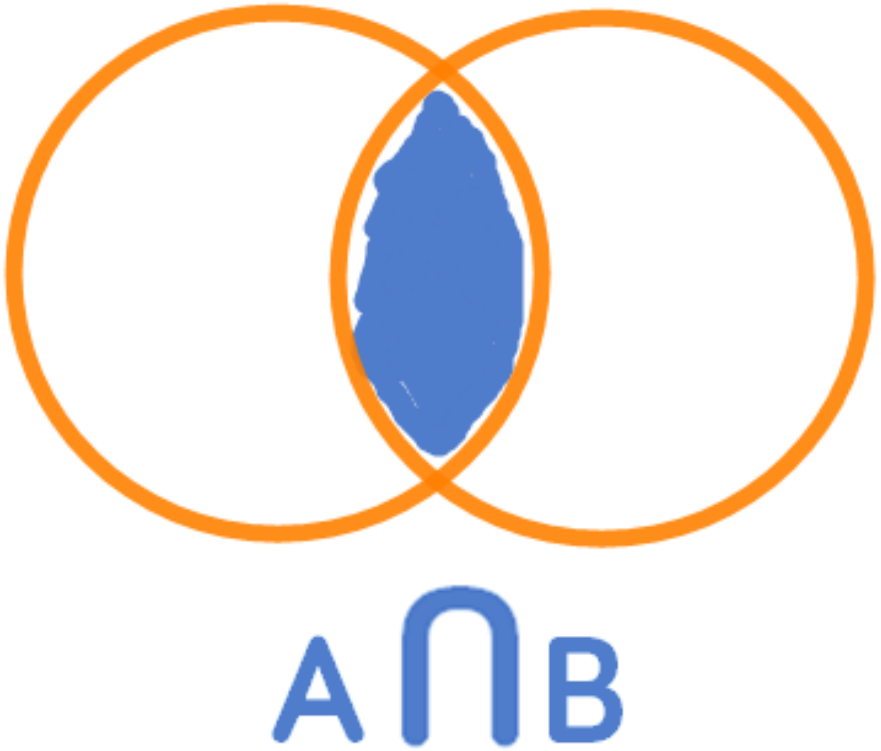
https://www.khanacademy.org/science/biology/her/tree-of-life/a/phylogenetic-trees

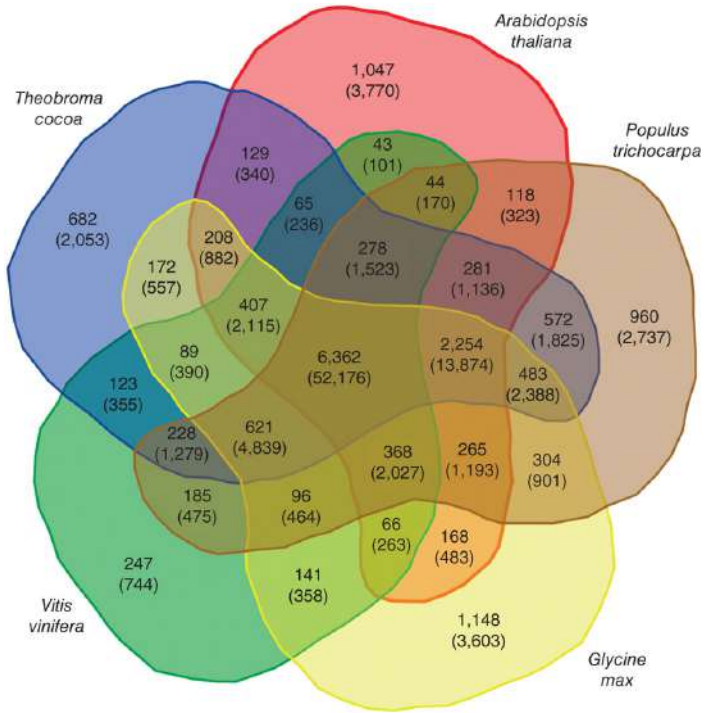
Phylogeny with added features

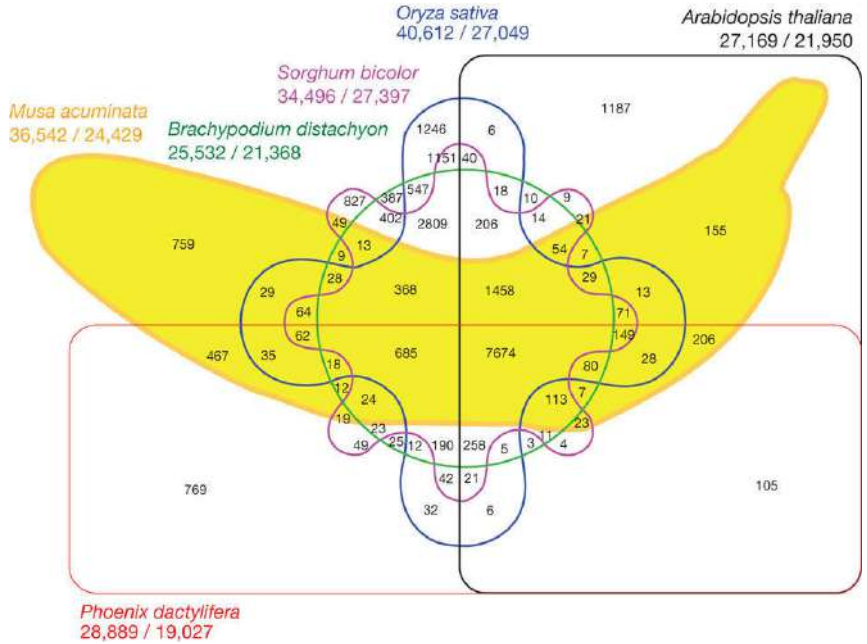


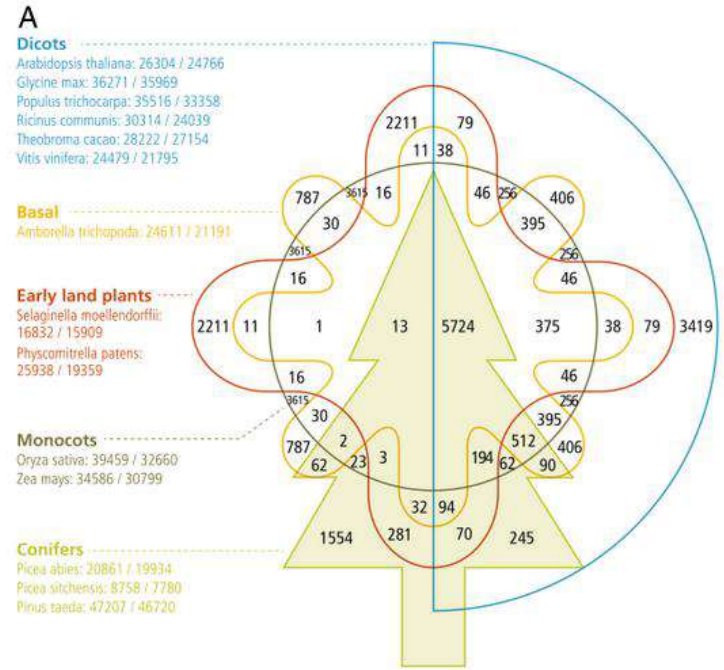
Nature Reviews | Genetics

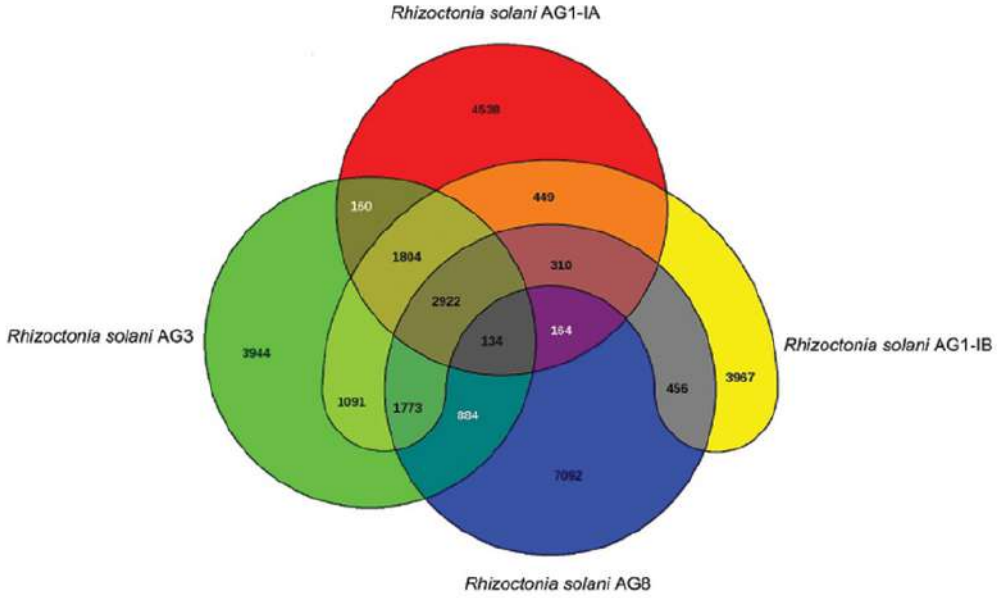
Intersections, unions – Venn diagrams




Arabidopsis thaliana
Theobroma cocoa
Populus trichocarpa
Vitis vinifera
Glycine max
1,047 (3,770)
682 (2,053)
129 (340)
43 (101)
65 (236)
44 (170)
118 (323)
208 (882)
278 (1,523)
281 (1,136)
172 (557)
407 (2,115)
572 (1,825)
960 (2,737)
89 (390)
6,362 (52,176)
2,254 (13,874)
483 (2,388)
123 (355)
228 (1,279)
621 (4,839)
368 (2,027)
265 (1,193)
304 (901)
185 (475)
96 (464)
66 (263)
168 (483)
247 (744)
141 (358)
1,148 (3,603)


Oryza sativa
40,612 / 27,049
Arabidopsis thaliana
27,169 / 21,950
Sorghum bicolor
34,496 / 27,397
Musa acuminata
36,542 / 24,429
Brachypodium distachyon
25,532 / 21,368
Phoenix dactylifera
28,889 / 19,027


A
Dicots
Arabidopsis thaliana: 26304 / 24766
Glycine max: 36271 / 35969
Populus trichocarpa: 35516 / 33358
Ricinus communis: 30314 / 24039
Theobroma cacao: 28222 / 27154
Vitis vinifera: 24479 / 21795
Basal
Amborella trichopoda: 24611 / 21191
Early land plants
Selaginella moellendorffii: 16832 / 15909
Physcomitrella patens: 25938 / 19359
Monocots
Oryza sativa: 39459 / 32660
Zea mays: 34586 / 30799
Conifers
Picea abies: 20861 / 19934
Picea sitchensis: 8758 / 7780
Pinus taeda: 47207 / 46720


Rhizoctonia solani AG1-IA
Rhizoctonia solani AG3
Rhizoctonia solani AG1-IB
Rhizoctonia solani AG8

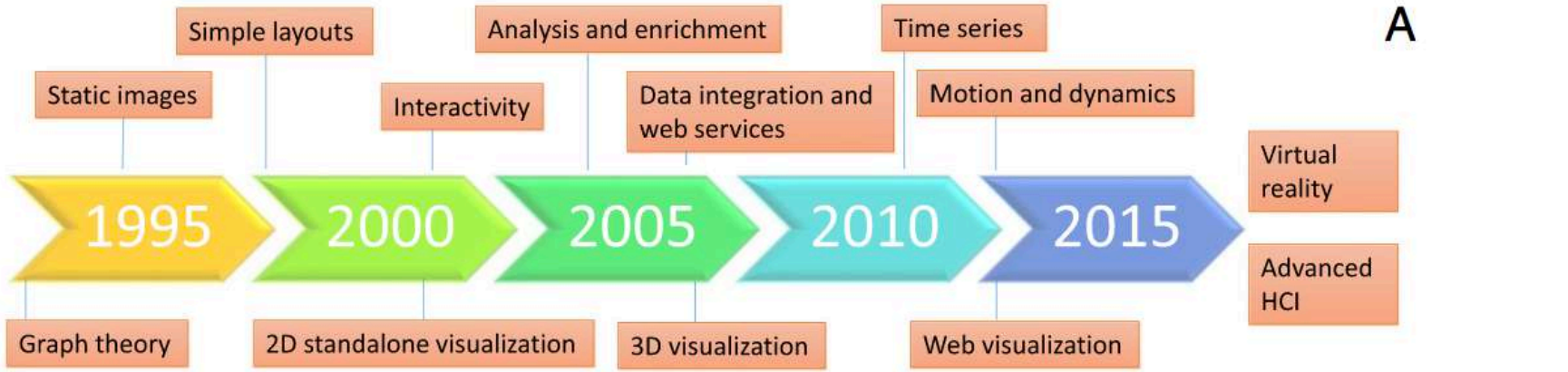
Pavlopoulos *et al. GigaScience* (2015) 4:38
DOI 10.1186/s13742-015-0077-2

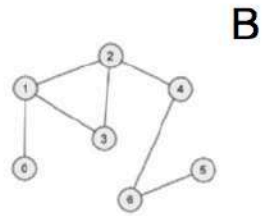
(GIGA)n SCIENCE

REVIEW Open Access

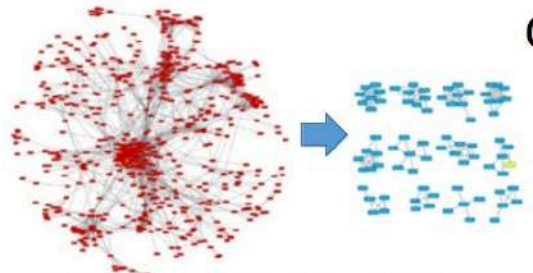
Visualizing genome and systems biology: technologies, tools, implementation techniques and trends, past, present and future

Georgios A. Pavlopoulos[1*], Dimitris Malliarakis[2], Nikolas Papanikolaou[1], Theodosis Theodosiou[1], Anton J. Enright[3] and Ioannis Iliopoulos[1*]

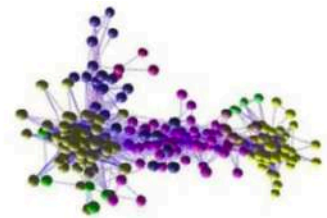




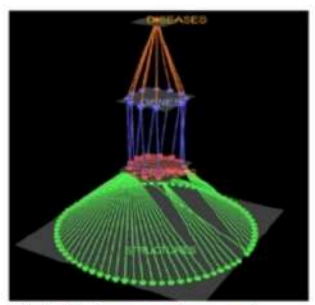
Simple graph



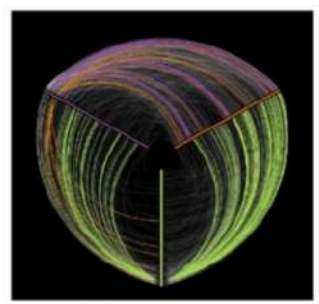
PPI network and protein complexes



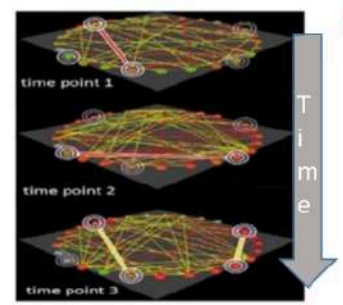
3D visualization



Multi-layered graphs

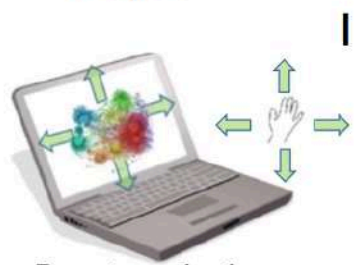


Hive plots

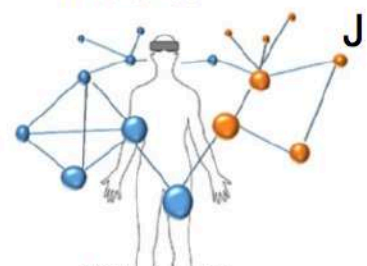


Time series

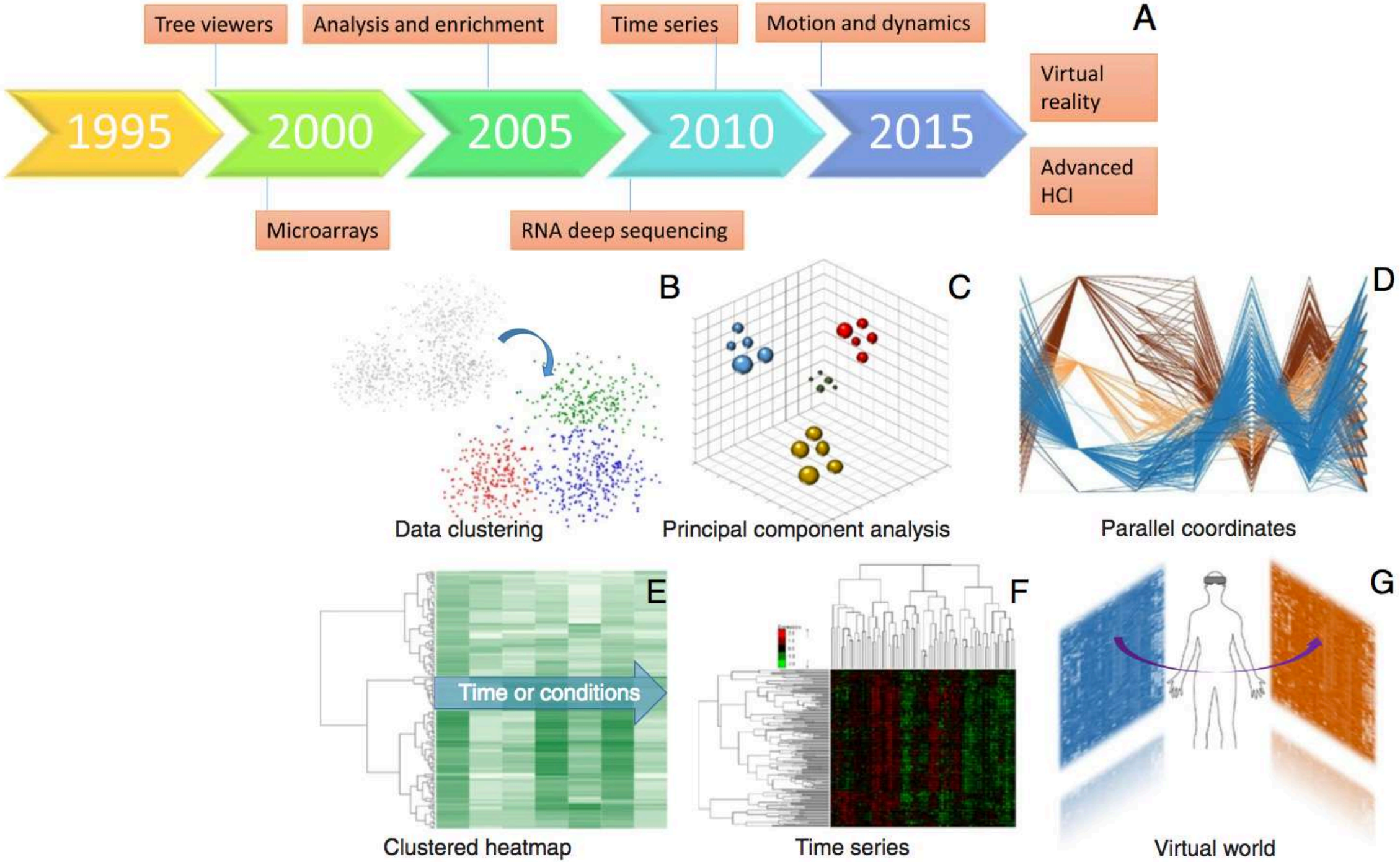
Pathway



Remote navigation



Virtual reality

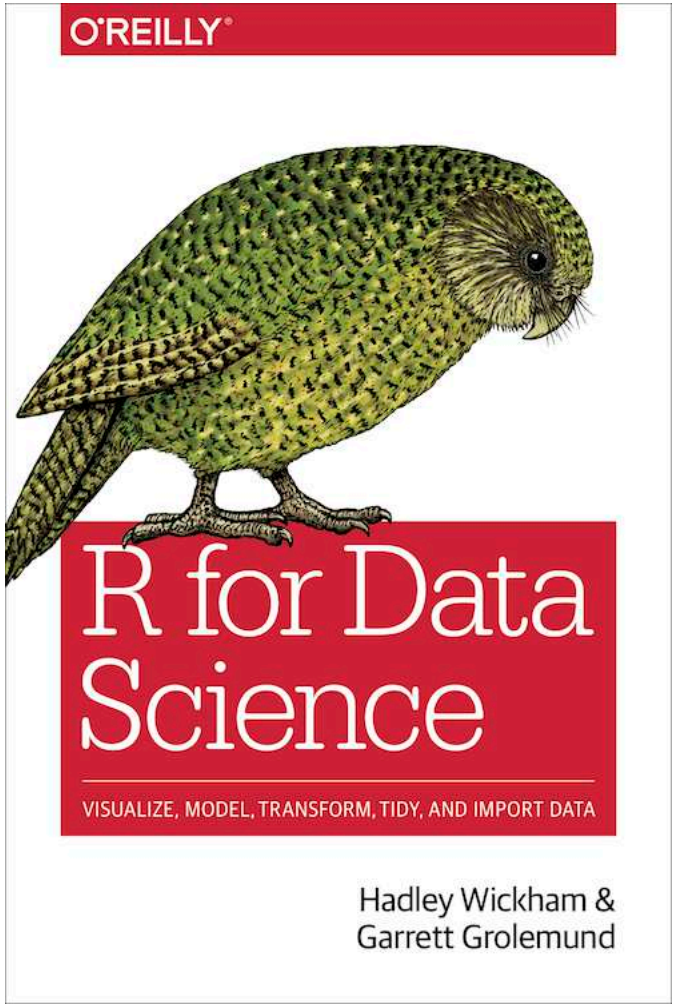

A
Tree viewers
Analysis and enrichment
Time series
Motion and dynamics
1995
2000
2005
2010
2015
Virtual reality
Advanced HCI
Microarrays
RNA deep sequencing
B
Data clustering
C
Principal component analysis
D
Parallel coordinates
E
Time or conditions
Clustered heatmap
F
Time series
G
Virtual world

R is a programming environment

- It's **free**
  - Hence R is supported by a large user network
  - R is open source
- Can be run on Windows, Linux and Mac
- Provides an unparalleled platform for programming new statistical methods in an easy and straightforward manner.
- **Excellent graphics capabilities**
- **Lots and lots of analysis packages**
- It is also **old**, hence you need to know new functions which do things much faster

Suggested textbook (also a gitbook!)

http://r4ds.had.co.nz/



R for Data Science

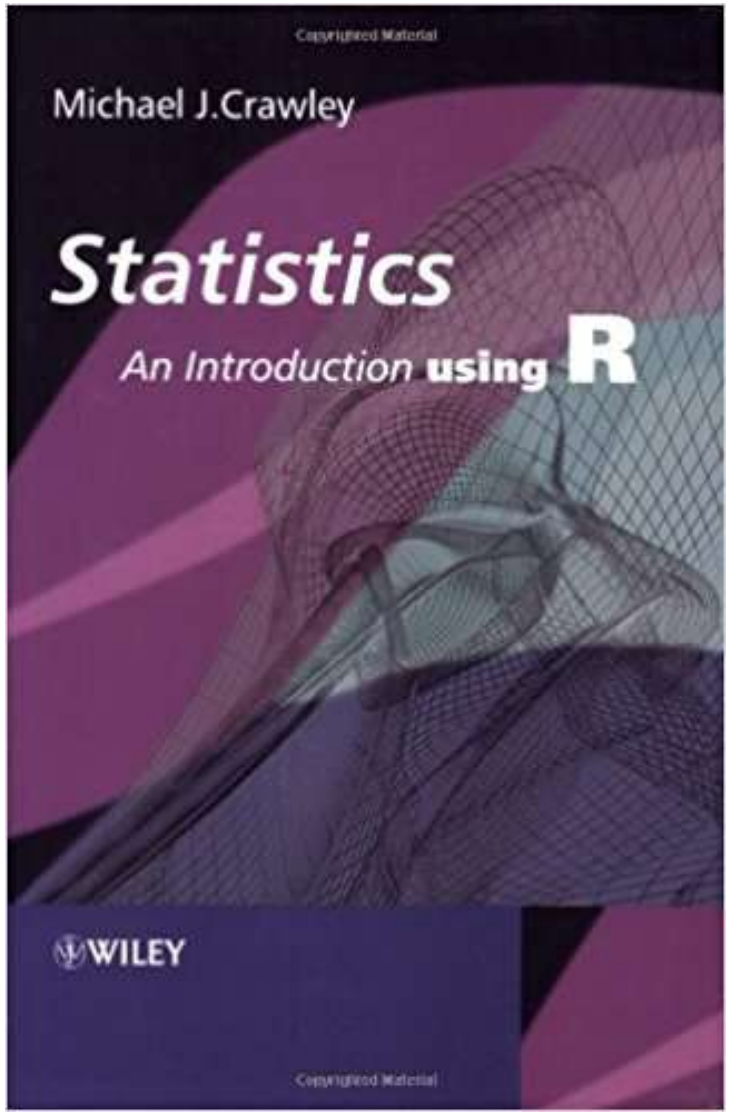
Garrett Grolemund

Hadley Wickham

Welcome

This is the website for **"R for Data Science"**. This book will teach you how to do data science with R: You'll learn how to get your data into R, get it into the most useful structure, transform it, visualise it and model it. In this book, you will find a practicum of skills for data science. Just as a chemist learns how to clean test tubes and stock a lab, you'll learn how to clean data and draw plots—and many other things besides. These are the skills that allow data science to happen, and here you will find the best practices for doing each of these things with R. You'll learn how to use the grammar of graphics, literate programming, and reproducible research to save time. You'll also learn how to manage cognitive resources to facilitate discoveries when wrangling, visualising, and exploring data.

Suggested textbook + learn statistics



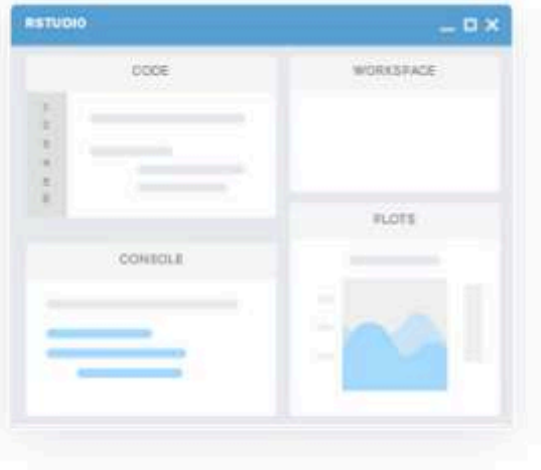
Code is kind of obsoleted but contents about statistics are still outstanding

http://onlinelibrary.wiley.com/book/10.1002/9781119941750

Download R and Rstudio

RStudio

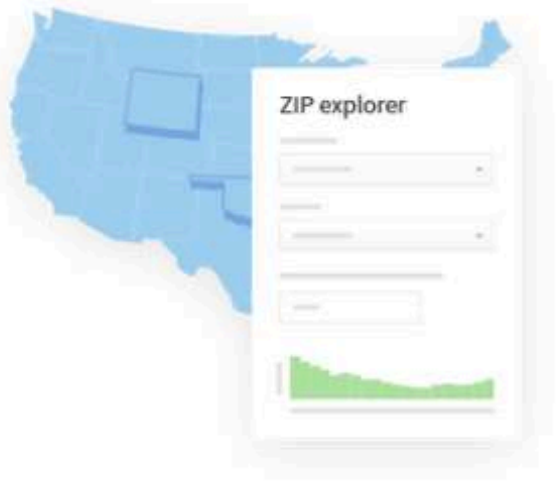
http://www.r-project.org
https://www.rstudio.com/



RStudio

RStudio makes R easier to use. It includes a code editor, debugging & visualization tools.

Download Learn More



Shiny

Shiny helps you make interactive web applications for visualizing data. Bring R data analysis to life.

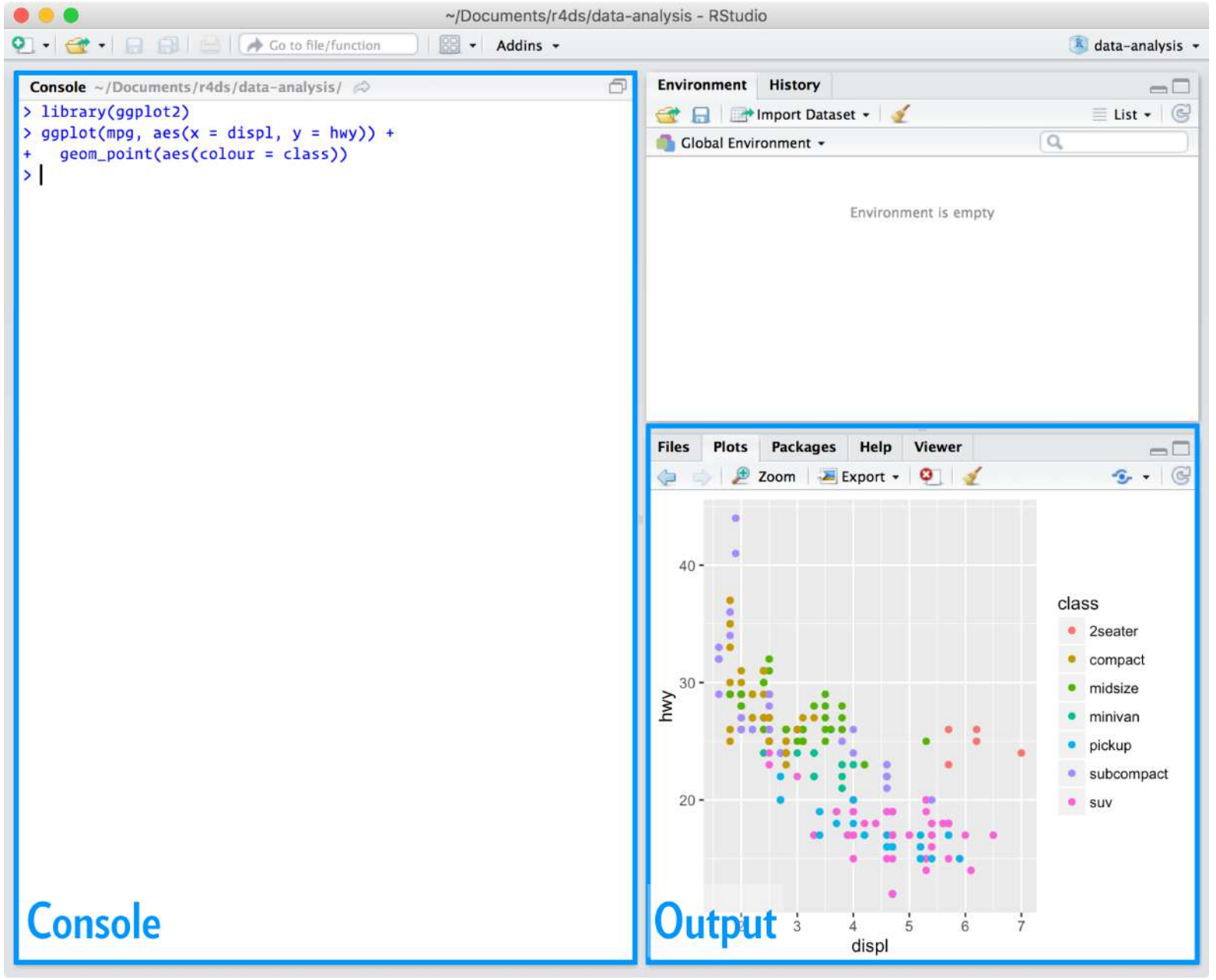
Learn More

R Packages

Our developers create popular packages to expand the features of R. Includes ggplot2, dplyr, R Markdown & more.

Learn More

Rstudio interface



R as a calculator

```
> 2+3
[1] 5
> 2*3
[1] 6
> 1
[1] 1
> 1 + 3
[1] 4
> 3 +
+ 1111 -
+ 1000
[1] 114
>
```

Press enter to complete the expression

Completed expression

Incomplete expression will result in continuation prompt **+**

Assignment

```
> x <- 5
> x
[1] 5
> y <- 10
> y
[1] 10
> x+y
[1] 15
> X <- 10
> X
[1] 10
> x
[1] 5
> x <- 100
> x
[1] 100
> z <- x + y + X
> z
[1] 120
```

<- is the assignment operation

R is case sensitive ; **x** does not equal to **X**

Original value is replaced

New value can be assigned as the result of calculation

Boolean assignment

```
student <- 30000
phd <- 56000
```

```
student > phd
```

```
[1] FALSE
```

```
student < phd
```

```
[1] TRUE
```

```
student != phd
```

```
[1] FALSE
```

```
student + student > phd
```

#Two heads are better than one

```
[1] TRUE
```

Vector is the simplest data structure in R

```
x<- c(1,2,3,4,5,6,7,8,9,10)
```

c = combine
In this case, we assign a **vector** of 10 numbers into x

```
x * 2
x /10 + 1
```

Selection

```
x<- c(1,2,3,4,5,6,7,8,9,10)
names(x)<-c("A","B","C","D","E","F","G","H","I","J")
```

```
x[x>5]
x[1:3]
x[1]
x[-1]
x[c("C","D")]
x[c("Z")]
x[x %in% c(7,9)]
x[x %in% c(7,13)]
```

```
> x[c("C","D")]
C D
3 4
> x[c("Z")]
<NA>
  NA
```

```
> x[x %in% 5]
E
5
> x[x %in% 10]
 J
10
> x[x %in% c(7,9)]
G I
7 9
> x[x %in% c(7,13)]
G
7
```

```
> x[x>5]
 F  G  H  I  J
 6  7  8  9 10
> x[1:3]
A B C
1 2 3
> x[1]
A
1
> x[-1]
 B  C  D  E  F  G  H  I  J
 2  3  4  5  6  7  8  9 10
```

Different types of vectors

```
x<- c(1,2,3,4,5,6,7,8,9,10)
strings <- c("AS","BRC")

typeof(x)
typeof(strings)
```

```
> typeof(x)
[1] "double"
> typeof(char)
[1] "character"
> typeof(strings)
[1] "character"
```

This matters when one data type is numbers, and you want to sort them categorically

Function

function (arg1, arg2, arg3… , option1=,option2=...)

```
x<- c(1,2,3,4,5,6,7,8,9,10)
y<- c(3,6,9,10,13,30,20,100)
```

```
mean(x)
mean(y)
median(x)
max(x)
```

```
> x<- c(1,2,2,3,5,6,7,10)
> y<- c(3,6,9,10,13,30,20,100)
> mean(x)
[1] 4.5
> mean(y)
[1] 23.875
> median(x)
[1] 4
> median(y)
[1] 11.5
> max(x)
[1] 10
> min(y)
[1] 3
```

- Must have **assigned names**
- Applies using **round brackets**
- Takes **argument** and options

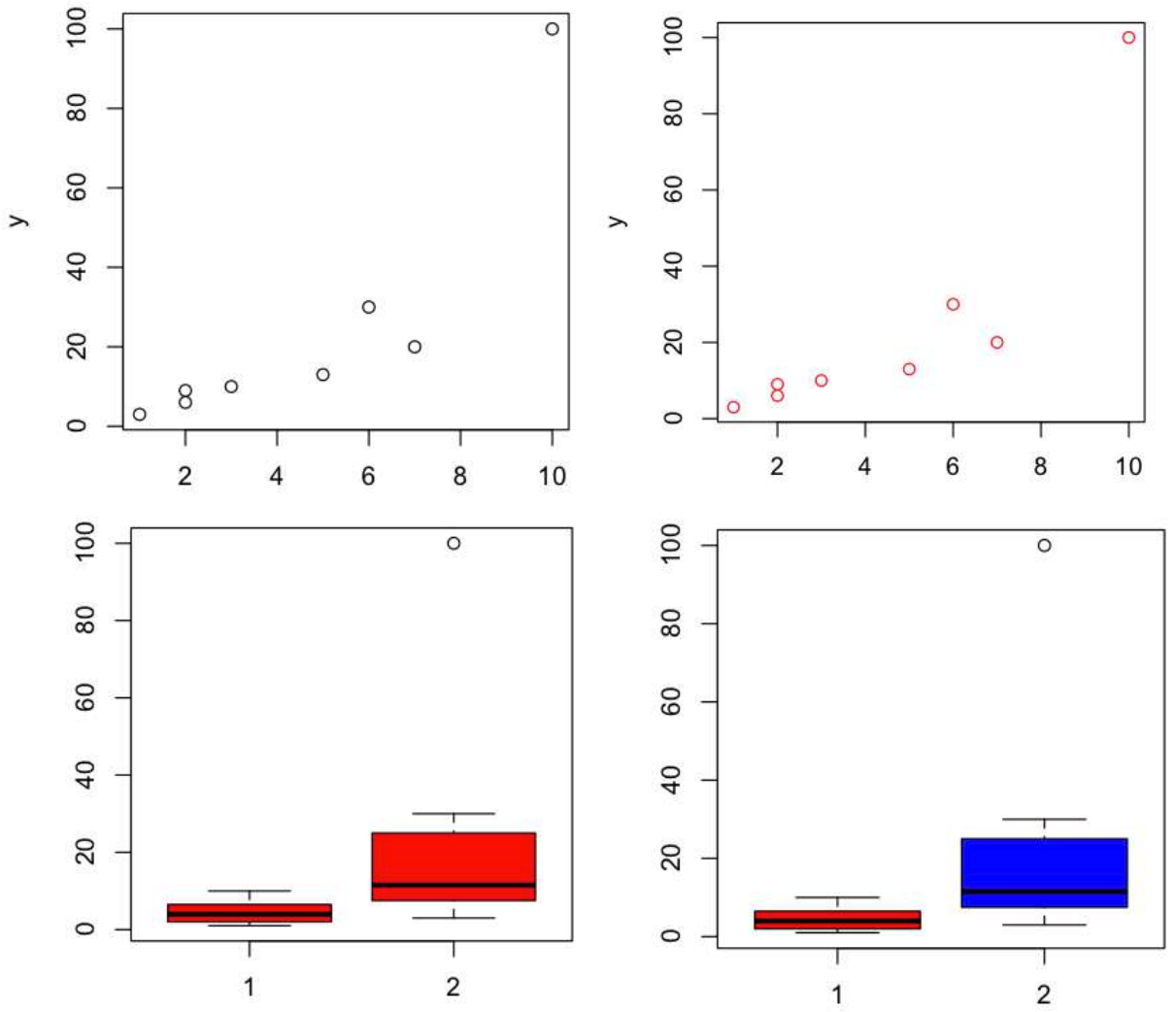
R simple plot I

```
x<- c(1,2,3,4,5,6,7,8,9,10)
y<- c(3,6,9,10,13,30,20,100,220,100)

plot(x,y)
plot(x,y,col="red")

boxplot(x,y,col="red")
boxplot(x,y,col=c("hotpink", "yellow"))

boxplot(x,y,col=c("hotpink", "yellow"),main="Lec2")
```



R simple plot II

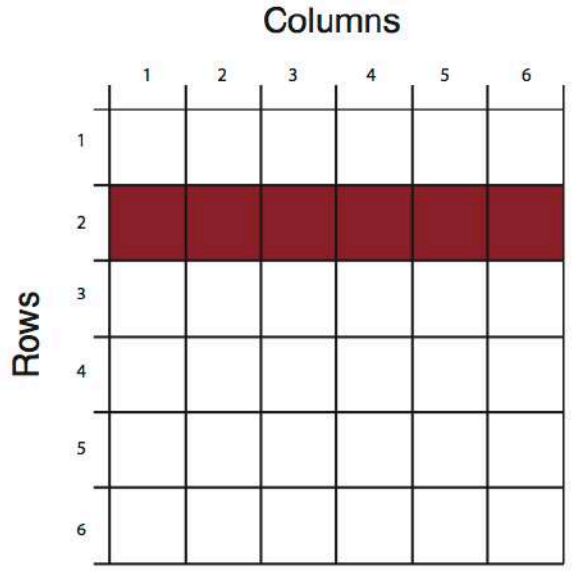
Follow examples here:
http://al2na.github.io/compgenr/intro_to_r/plotting_in_r.html

Matrices are a collection of vectors of the same type

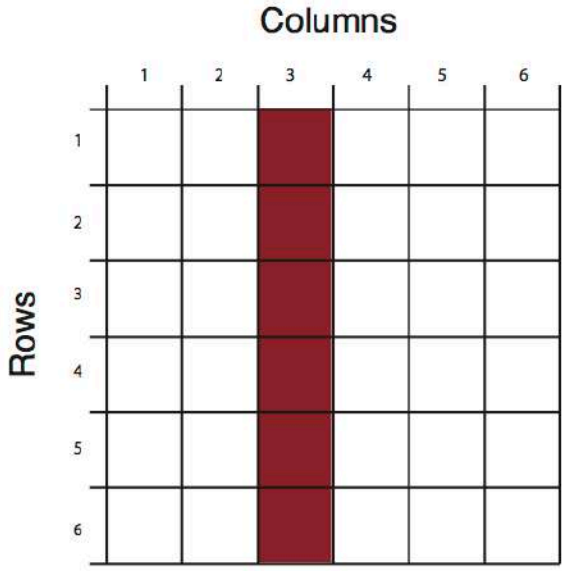
```
mat <- matrix(c(1, 3, 2, 5, -1, 2, 2, 3, 9), nrow = 3)
rownames(mat) <- c("a","b","c")
colnames(mat) <- c(”x",”y",”z")
```

```
  [,1] [,2] [,3]
a    1    5    2
b    3   -1    3
c    2    2    9
```

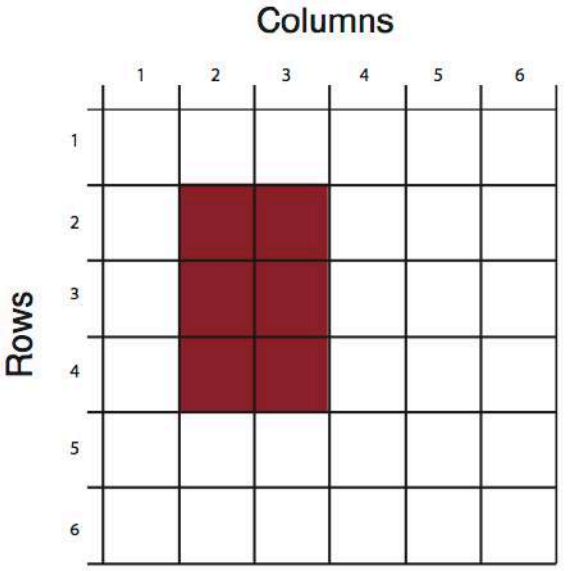
`mat[2,]`



`mat[,3]`



`mat[2:4,2:3]`



http://al2na.github.io/compgenr/intro_to_r/data_types.html

Matrices - summary

- Each row and column must have data of the **same** type (numeric, character etc)
- Most useful when do linear algebra (e.g. PCA,)

```
> mat * 2
     [,1] [,2] [,3]
[1,]    2   10    4
[2,]    6   -2    6
[3,]    4    4   18
```

- If you want **different** data types, need to use objects called data.frames

Data frames

- Think of these like Excel spreadsheets
- **All the values of the same variable must go in the same column**
  - E.g., age, sex, RPKM, numbers
- **Rows represent samples**
  - E.g., sample A collected in Taiwan, sample B collected in Japan

- Like matrices but different types of data are allowed

- Tibble from the **dplyr** package ; basically like data frame but much easier to manipulate

R has some pre-installed data frames

```
iris
head(iris)
```

Or you can read into data

```
worms <- read.table("worms.txt", header=T)
head(worms)
```

```
> worms
          Field.Name Area Slope Vegetation Soil.pH  Damp Worm.density
1        Nashs.Field  3.6    11  Grassland     4.1 FALSE            4
2     Silwood.Bottom  5.1     2     Arable     5.2 FALSE            7
3      Nursery.Field  2.8     3  Grassland     4.3 FALSE            2
4       Rush.Meadow  2.4     5     Meadow     4.9  TRUE            5
5    Gunness.Thicket  3.8     0      Scrub     4.2 FALSE            6
6           Oak.Mead  3.1     2  Grassland     3.9 FALSE            2
7       Church.Field  3.5     3  Grassland     4.2 FALSE            3
8            Ashurst  2.1     0     Arable     4.8 FALSE            4
9       The.Orchard  1.9     0    Orchard     5.7 FALSE            9
10     Rookery.Slope  1.5     4  Grassland     5.0  TRUE            7
11      Garden.Wood  2.9    10      Scrub     5.2 FALSE            8
12      North.Gravel  3.3     1  Grassland     4.1 FALSE            1
13      South.Gravel  3.7     2  Grassland     4.0 FALSE            2
14 Observatory.Ridge  1.8     6  Grassland     3.8 FALSE            0
15        Pond.Field  4.1     0     Meadow     5.0  TRUE            6
16      Water.Meadow  3.9     0     Meadow     4.9  TRUE            8
17         Cheapside  2.2     8      Scrub     4.7  TRUE            4
18        Pound.Hill  4.4     2     Arable     4.5 FALSE            5
19        Gravel.Pit  2.9     1  Grassland     3.5 FALSE            1
20         Farm.Wood  0.8    10      Scrub     5.1  TRUE            3
```

File available here:
https://github.com/shifteight/R/blob/master/TRB/data/worms.txt

Selection in data frames

Square brackets

- **dat[i ,]** would select the *i*-th row (which is a **vector**)
- **dat[, j]** would select the *j*-th column (which is a **vector**)
- **dat[i, j]** would select the value from the *i*-th row and *j*-th column

```
worms[,1]
worms[1,]
worms[1,1]
```

dollar ($) operation (for columns only)

```
worms$Area
```

subset (not discussing today)

Some combinations of it

Square brackets

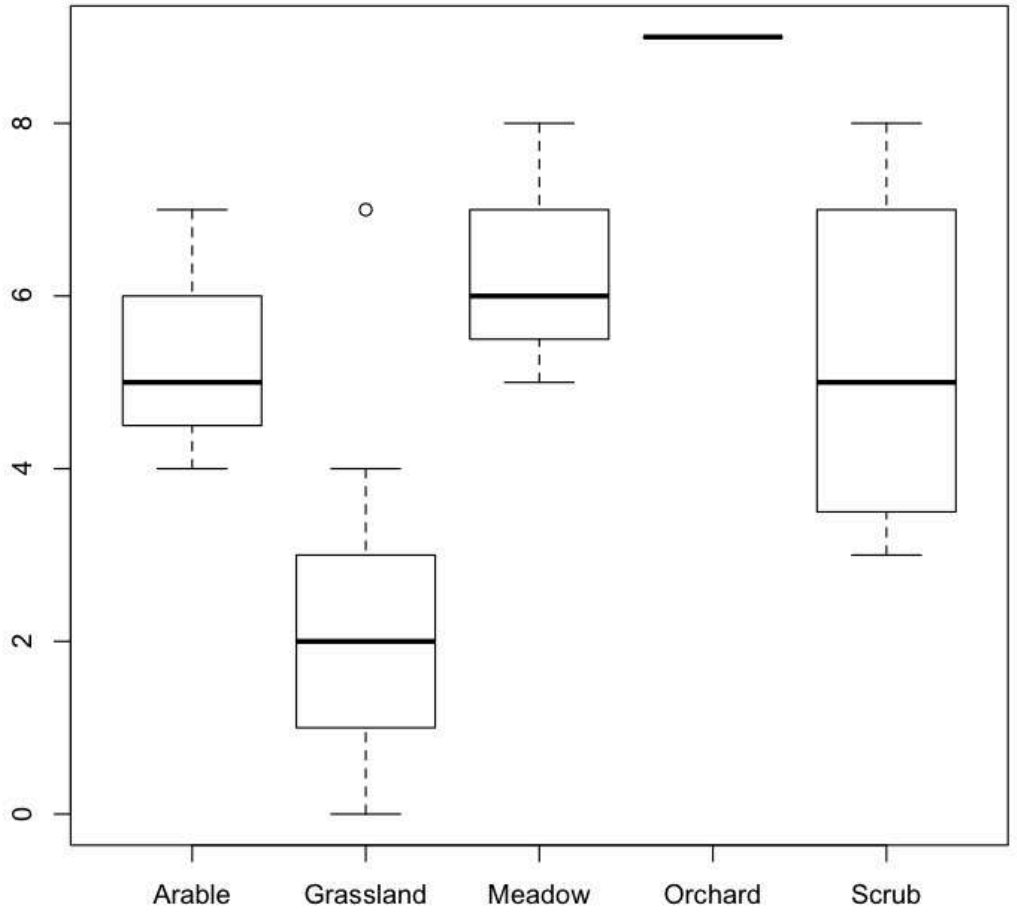
- **dat[i ,]** would select the *i*-th row (which is a **vector**)
- **dat[, j]** would select the *j*-th column (which is a **vector**)
- **dat[i, j]** would select the value from the *i*-th row and *j*-th column

```
worms[worms$Area < 3,]
worms[(worms$Area < 3) & (worms$Worm.density <4),]
worms[(worms$Area < 3) & (worms$Worm.density <4),]$Soil.pH
```

```
> worms
          Field.Name Area Slope Vegetation Soil.pH  Damp Worm.density
1        Nashs.Field  3.6    11  Grassland     4.1 FALSE            4
2     Silwood.Bottom  5.1     2     Arable     5.2 FALSE            7
3      Nursery.Field  2.8     3  Grassland     4.3 FALSE            2
4       Rush.Meadow  2.4     5     Meadow     4.9  TRUE            5
5    Gunness.Thicket  3.8     0      Scrub     4.2 FALSE            6
6           Oak.Mead  3.1     2  Grassland     3.9 FALSE            2
7       Church.Field  3.5     3  Grassland     4.2 FALSE            3
8            Ashurst  2.1     0     Arable     4.8 FALSE            4
9       The.Orchard  1.9     0    Orchard     5.7 FALSE            9
10     Rookery.Slope  1.5     4  Grassland     5.0  TRUE            7
11      Garden.Wood  2.9    10      Scrub     5.2 FALSE            8
12     North.Gravel  3.3     1  Grassland     4.1 FALSE            1
13     South.Gravel  3.7     2  Grassland     4.0 FALSE            2
14 Observatory.Ridge  1.8     6  Grassland     3.8 FALSE            0
15       Pond.Field  4.1     0     Meadow     5.0  TRUE            6
16     Water.Meadow  3.9     0     Meadow     4.9  TRUE            8
17        Cheapside  2.2     8      Scrub     4.7  TRUE            4
18       Pound.Hill  4.4     2     Arable     4.5 FALSE            5
19       Gravel.Pit  2.9     1  Grassland     3.5 FALSE            1
20        Farm.Wood  0.8    10      Scrub     5.1  TRUE            3
```

More plot from dataframes

```
plot(worms$Area,worms$Slope,col=as.numeric(worms$Vegetation))
plot(worms$Area,worms$Slope,col=as.numeric(worms$Vegetation),pch=as.numeric(worms$Vegetation))
boxplot(worms$Worm.density ~ worms$Vegetation)
```




```
> worms
          Field.Name Area Slope Vegetation Soil.pH  Damp Worm.density
1        Nashs.Field  3.6    11  Grassland     4.1 FALSE            4
2     Silwood.Bottom  5.1     2     Arable     5.2 FALSE            7
3      Nursery.Field  2.8     3  Grassland     4.3 FALSE            2
4       Rush.Meadow  2.4     5     Meadow     4.9  TRUE            5
5    Gunness.Thicket  3.8     0      Scrub     4.2 FALSE            6
6           Oak.Mead  3.1     2  Grassland     3.9 FALSE            2
7       Church.Field  3.5     3  Grassland     4.2 FALSE            3
8            Ashurst  2.1     0     Arable     4.8 FALSE            4
9        The.Orchard  1.9     0    Orchard     5.7 FALSE            9
10     Rookery.Slope  1.5     4  Grassland     5.0  TRUE            7
11      Garden.Wood  2.9    10      Scrub     5.2 FALSE            8
12     North.Gravel  3.3     1  Grassland     4.1 FALSE            1
13     South.Gravel  3.7     2  Grassland     4.0 FALSE            2
14 Observatory.Ridge  1.8     6  Grassland     3.8 FALSE            0
15       Pond.Field  4.1     0     Meadow     5.0  TRUE            6
16     Water.Meadow  3.9     0     Meadow     4.9  TRUE            8
17        Cheapside  2.2     8      Scrub     4.7  TRUE            4
18       Pound.Hill  4.4     2     Arable     4.5 FALSE            5
19       Gravel.Pit  2.9     1  Grassland     3.5 FALSE            1
20        Farm.Wood  0.8    10      Scrub     5.1  TRUE            3
```

More useful functions here

```
y<-abs(-20)
x<-Sum(y+5)
Z<-Log(x)
round(x,1)
summary(worms)
head(worms)
tail(worms)
ncol(worms)
nrow(worms)
```

Statistics

Simulate two normal distributions one at mean =4, and another at 6

```
x <- rnorm(500,4)                    # mean at 4
y <- rnorm(500,6)                    # mean at 6


# Plot histogram

plot(hist(x), col=rgb(0,0,1,1/4), xlim=c(0,10))
plot(hist(y), col=rgb(1,0,0,1/4), xlim=c(0,10), add=T)

t.test(x,y)
```

Simulate two normal distributions at mean =3

```
x <- rnorm(500,3)
y <- rnorm(500,3)
t.test(x,y)
```

Running out of functions to use?

Use Packages

- R consists of a **core** and **additional packages**.
- Collections of R functions, data, and compiled code
- Well-defined format that ensures easy installation, a basic standard of documentation, and enhances portability and reliability

Install R packages

You'll also need to install some R packages. An R **package** is a collection of functions, data, and documentation that extends the capabilities of base R. Using packages is key to the successful use of R. The majority of the packages that you will learn in this book are part of the so-called tidyverse. The packages in the tidyverse share a common philosophy of data and R programming, and are designed to work together naturally.

You can install the complete tidyverse with a single line of code:

```
install.packages("tidyverse")
```

http://r4ds.had.co.nz/introduction.html#rstudio

Tidyverse package

Tidyverse

Packages Articles Learn Help Contribute

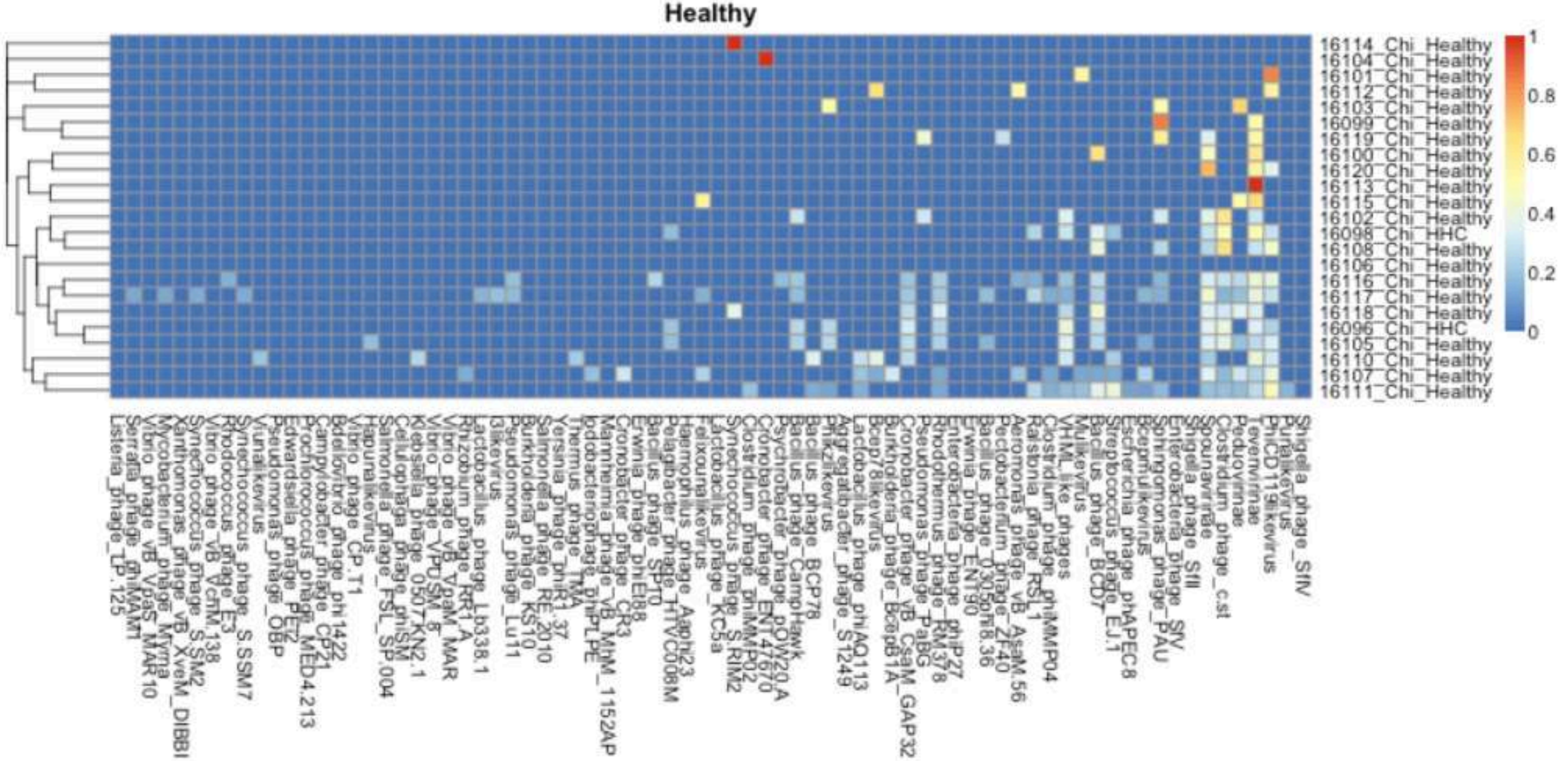
R packages for data science

The tidyverse is an opinionated **collection of R packages** designed for data science. All packages share an underlying design philosophy, grammar, and data structures.

Install the complete tidyverse with:

```
install.packages("tidyverse")
```

Example I




```
library("pheatmap")
library("vegan")
healthy <- read.table(“myoviridae_healthy.txt")
healthy_hellinger <- decostand(healthy, method="hellinger")
pheatmap(healthy_hellinger, cluster_cols=FALSE, cellwidth=8, cellheight=8, main="Healthy")
```

Case study one (iris)

The data set consists of **50 samples from each of three species of Iris (Iris setosa, Iris virginica and Iris versicolor).** Four features were measured from each sample: the **length and the width of the sepals and petals, in centimetres**. Based on the combination of these four features, Fisher developed a linear discriminant model to distinguish the species from each other.

This data set became a typical test case for many statistical classification techniques in machine learning such as support vector machines

Iris setosa

Iris versicolor

Iris virginica

THE USE OF MULTIPLE MEASUREMENTS IN TAXONOMIC PROBLEMS

By R. A. FISHER, Sc.D., F.R.S.

I. Discriminant functions

When two or more populations have been measured in several characters, $x_1, \ldots, x_p$, special interest attaches to certain linear functions of the measurements by which the populations are best discriminated. At the author's suggestion use has already been made of this fact in craniometry (*a*) by Mr E. S. Martin, who has applied the principle to the sex differences in measurements of the mandible, and (*b*) by Miss Mildred Barnard, who showed how to obtain from a series of dated series the particular compound of cranial measurements showing most distinctly a progressive or secular trend. In the present paper the application of the same principle will be illustrated on a taxonomic problem; some questions connected with the precision of the processes employed will also be discussed.

II. Arithmetical procedure

Table I shows measurements of the flowers of fifty plants each of the two species *Iris setosa* and *I. versicolor*, found growing together in the same colony and measured by Dr E. Anderson, to whom I am indebted for the use of the data. Four flower measurements are given. We shall first consider the question: What linear function of the four measurements $X = \lambda_1 x_1 + \lambda_2 x_2 + \lambda_3 x_3 + \lambda_4 x_4$ will maximize the ratio of the difference between the specific means to the standard deviations within species? The observed means and their differences are shown in Table II. We may represent the differences by d_p, where $p = 1, 2, 3$ or 4 for the four measurements.

The sums of squares and products of deviations from the specific means are shown in Table III. Since fifty plants of each species were used these sums contain 98 degrees of freedom. We may represent these sums of squares or products by S_{pq}, where p and q take independently the values 1, 2, 3 and 4.

Then for any linear function, X, of the measurements, as defined above, the difference between the means of X in the two species is

$$D = \lambda_1 d_1 + \lambda_2 d_2 + \lambda_3 d_3 + \lambda_4 d_4,$$

while the variance of X within species is proportional to

$$S = \sum_{p=1}^{4} \sum_{q=1}^{4} \lambda_p \lambda_q S_{pq}.$$

The particular linear function which best discriminates the two species will be one for

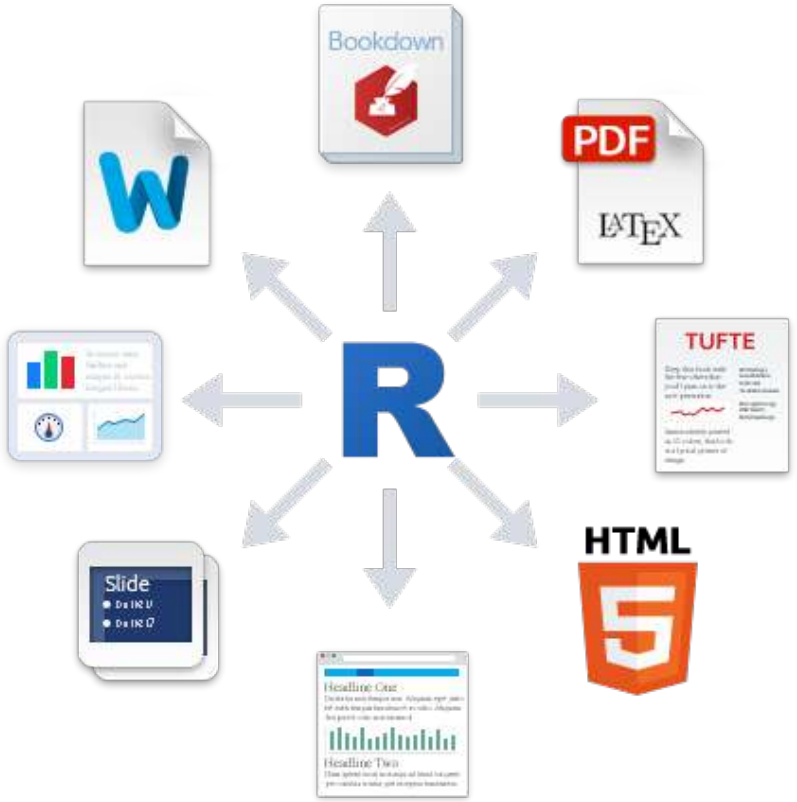
https://en.wikipedia.org/wiki/Iris_flower_data_set

Case study one (iris)

```
> iris
    Sepal.Length Sepal.Width Petal.Length Petal.Width    Species
1            5.1         3.5          1.4         0.2     setosa
2            4.9         3.0          1.4         0.2     setosa
3            4.7         3.2          1.3         0.2     setosa
4            4.6         3.1          1.5         0.2     setosa
5            5.0         3.6          1.4         0.2     setosa
6            5.4         3.9          1.7         0.4     setosa
7            4.6         3.4          1.4         0.3     setosa
8            5.0         3.4          1.5         0.2     setosa
9            4.4         2.9          1.4         0.2     setosa
10           4.9         3.1          1.5         0.1     setosa
11           5.4         3.7          1.5         0.2     setosa
12           4.8         3.4          1.6         0.2     setosa
13           4.8         3.0          1.4         0.1     setosa
14           4.3         3.0          1.1         0.1     setosa
15           5.8         4.0          1.2         0.2     setosa
16           5.7         4.4          1.5         0.4     setosa
17           5.4         3.9          1.3         0.4     setosa
18           5.1         3.5          1.4         0.3     setosa
```

https://rpubs.com/koushikstat/167274

R markdown in Rstudio



chunks.Rmd

Knit HTML | Chunks

```
1  R Code Chunks
2  ==========
3
4  With R Markdown, you can insert R code
   chunks including plots:
5
6  ```{r qplot, fig.width=4, fig.height=3,
   message=FALSE}
7  # quick summary and plot
8  library(ggplot2)
9  summary(cars)
10 qplot(speed, dist, data=cars) +
11       geom_smooth()
12 ```
13
```

RStudio: Preview HTML

Preview: ~/chunks.html | Save As | Publish

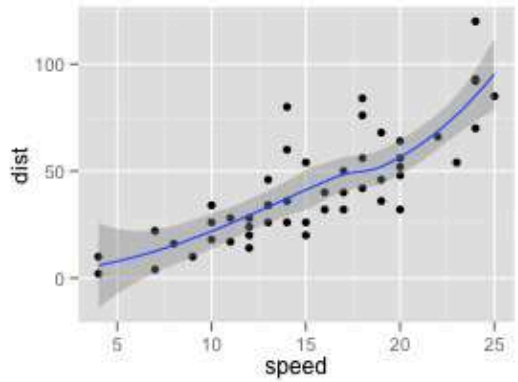
R Code Chunks

With R Markdown, you can insert R code chunks including plots:

```
# quick summary and plot
library(ggplot2)
summary(cars)
```

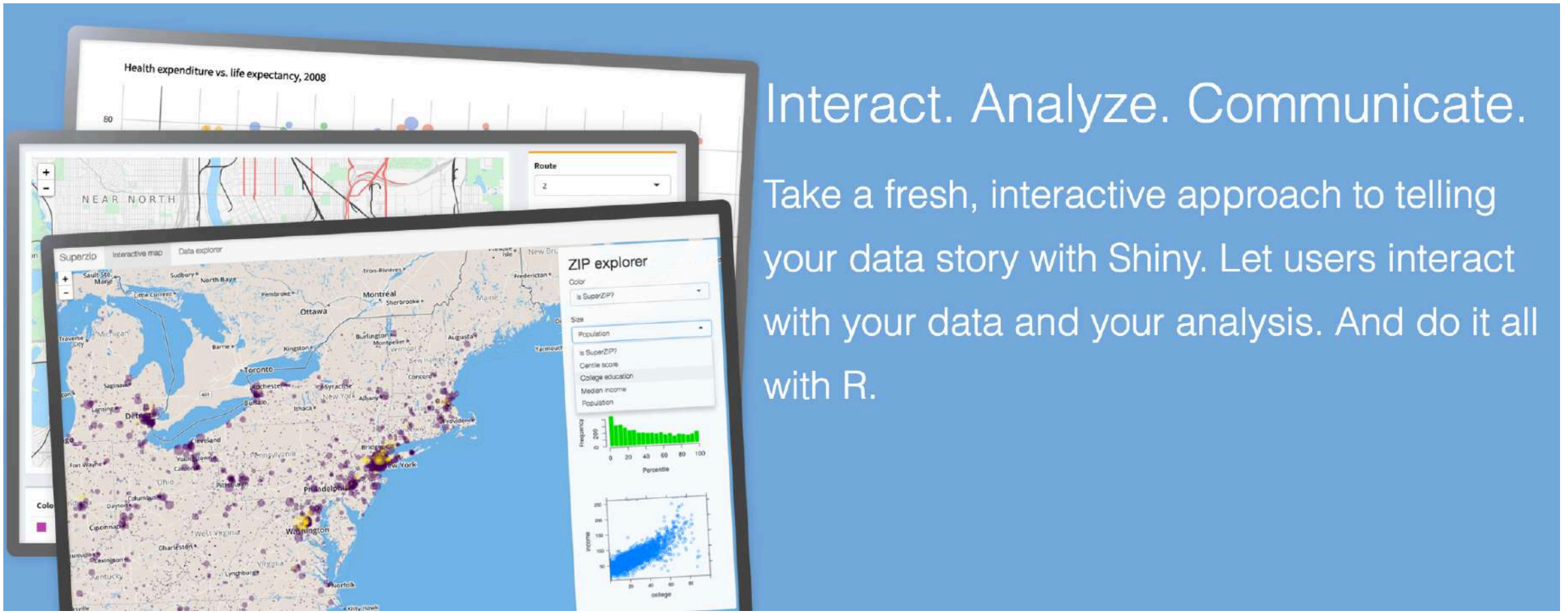
```
##      speed           dist
##  Min.   : 4.0   Min.   :  2
##  1st Qu.:12.0   1st Qu.: 26
##  Median :15.0   Median : 36
##  Mean   :15.4   Mean   : 43
##  3rd Qu.:19.0   3rd Qu.: 56
##  Max.   :25.0   Max.   :120
```

```
qplot(speed, dist, data = cars) + geom_smooth()
```



https://rmarkdown.rstudio.com/r_notebooks.html

Shiny from RStudio



Interact. Analyze. Communicate.

Take a fresh, interactive approach to telling your data story with Shiny. Let users interact with your data and your analysis. And do it all with R.

https://gallery.shinyapps.io/001-hello/
https://shiny.rstudio.com/gallery/genome-browser.html
https://shiny.rstudio.com/gallery/

Coronavirus examples

An R Package to Explore the Novel Coronavirus

Patrick Tung Follow

Feb 11 · 11 min read ★

https://towardsdatascience.com/an-r-package-to-explore-the-novel-coronavirus-590055738ad6

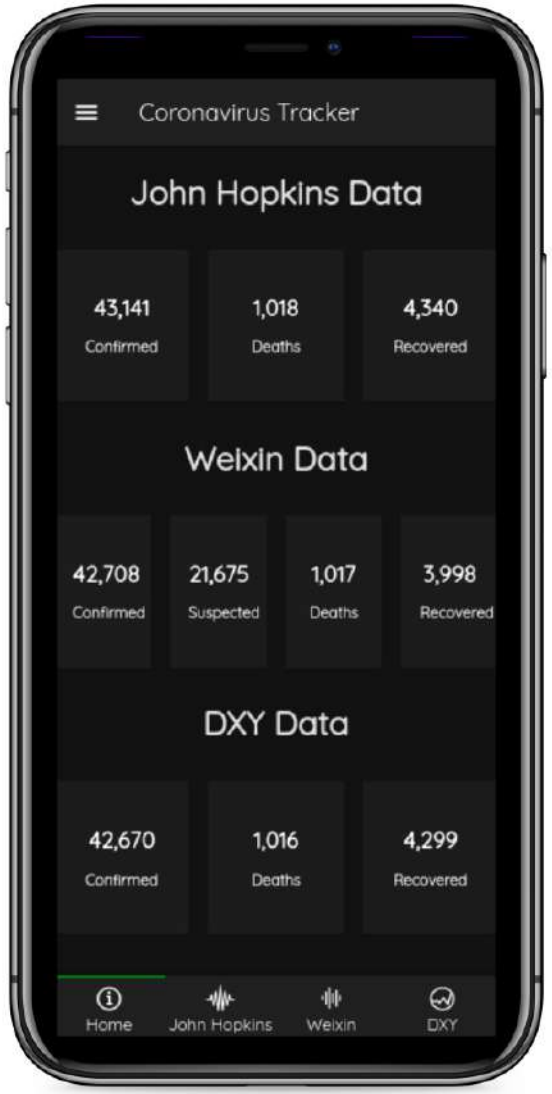
检索疫情数据的R包来了！

原创 Y叔叔 biobabble 2月3日

https://mp.weixin.qq.com/s/bPXdOGFzFK5dWLTEOEJB3g

https://mp.weixin.qq.com/s/_0D8ENb-4lGm4UV16Ok28A

Coronavirus Shiny example

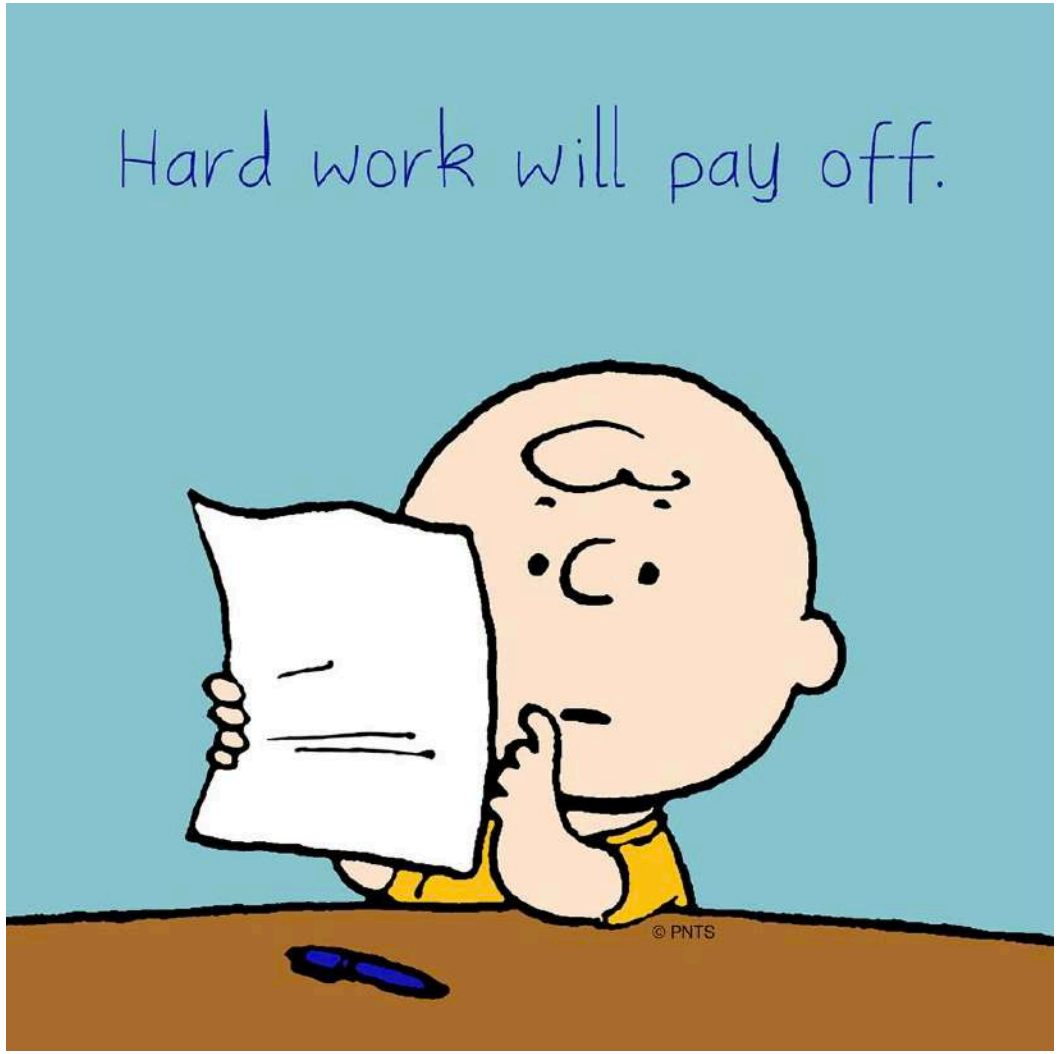


https://johncoene.shinyapps.io/contest-coronavirus/_w_5602e0c8/
https://community.rstudio.com/t/coronavirus-2020-shiny-contest-submission/53061

In summary

- Start practicing
- There are so much data out there
- Going through tutorials
- **Learn through real case scenarios**

- Think how to manage your notes and data effectively
- Research fast
- Reproducible research



Data sources

- https://data.gov.tw/ 

- http://fivethirtyeight.com/ FiveThirtyEight
- https://www.kaggle.com/ kaggle

- All the various R datasets:
  - https://vincentarelbundock.github.io/Rdatasets/datasets.html
  - Iris is part of them