INTRODUCTION

COURSE: GOOD PRACTICES FOR REPRODUCIBLE BIOINFORMATICS DATA ANALYSIS

Frédéric Lemoine 2021/05/23 Institut Pasteur





Module organization



Progress

- Monday 23th May
 - AM: Introduction to reproducibility. Managing code with git
 - PM: Practical about git
- Tuesday 24th May
 - AM: 1) Controling software environment with Conda
 - AM: 2) Controling software environment with containers (Singularity/Docker)
 - PM: Practical about Conda and containers
- Monday 30th May
 - AM: 1) Running data analyses with Notebooks
 - AM: 2) Running data analyses with workflows
 - PM: Practical about Workflows
- Tuesday 31th May
 - AM: Good practices for software, tools and script development
 - PM: Finalizing group projects + presentations of results



Team



Amandine Perrin, IR Pasteur (Hub Bioinfo, GEM unit)



Etienne Kornobis, IR Pasteur (Hub Bioinfo, Biomics platform)



Bertrand Néron, IR Pasteur (Hub Bioinfo, ALPS group)



Frédéric Lemoine, IR Pasteur (Hub Bioinfo, GEVA unit)



Project: Analyzing SARS-CoV-2 data

- Goal: Writing a reproducible workflow to analyse SARS-CoV-2 sequencing data:
 - 1. Mapping
 - 2. SNP Calling
 - 3. Consensus
 - 4. Clade detection
- **Using:**
 - 1. git
 - 2. containers
 - 3. 1 workflow system
- By groups of 4 people
- A final presentation the last day



"Hardware": Virtual machines from IT Dept

We will see that this afternoon but:

- 1. Connect to desktop.pasteur.fr
- 2. Go to the HTML access
- 3. Login with your Pasteur IDs
- 4. You are running a Linux Ubuntu VM with:
 - Singularity
 - Docker
 - Java
 - **♂** Conda
 - etc.





Introduction about reproducibility



History

In the past decade: Lots of debates around reproducibility.

Some fields that were particularly affected: Social sciences, Psychology, Clinical research;



Psychological research: Priming

Power of Suggestion

(https://www.chronicle.com/article/Power-of-Suggestion/136907/):

The studies that raise eyebrows are mostly in an area known as behavioral or goal priming, research that demonstrates how subliminal prompts can make you do all manner of crazy things. A warm mug makes you friendlier. The American flag makes you vote Republican. Fast-food logos make you impatient.

Priming: the original study

Cited > 5000 times (google scholar, 06/11/2019)!



Journal Information Journal TOC

Search APA PsycNET

PsycARTICLES: Journal Article

Automaticity of social behavior: Direct effects of trait construct and stereotype activation on action.

© Request Permissions

Bargh, John A., Chen, Mark, Burrows, Lara

Journal of Personality and Social Psychology, Vol 71(2), Aug 1996, 230-244

Previous research has shown that trait concepts and stereotypes become active automatically in the presence of relevant behavior or stereotyped-group features. Through the use of the same priming procedures as in previous impression formation research, Experiment 1 showed that participants whose concept of rudeness was primed interrupted the experimenter more quickly and frequently than did participants primed with polite-related stimuli. In Experiment 2, participants for whom an elderly stereotype was primed walked more slowly down the hallway when leaving the experiment than did control participants, consistent with the content of that stereotype. In Experiment 3, participants for whom the African American stereotype was primed subliminally reacted with more hostility to a vexatious request of the experimenter. Implications of this automatic behavior priming effect for self-fulfilling prophecies are discussed, as is whether social behavior is necessarily mediated by conscious choice processes. (PsycINFO Database Record (c) 2016 APA, all rights reserved)



Priming: Replication studies

Priming of Social Distance? Failure to Replicate Effects on Social and Food Judgments

Harold Pashler , Noriko Coburn, Christine R. Harris

Published: August 29, 2012 • https://doi.org/10.1371/journal.pone.0042510

Article	Authors	Metrics	Comments	Media Coverage
*				

Abstract

Study 1

Study 2

Discussion

Acknowledgments

Author Contributions

References

Abstract

Williams and Bargh (2008) reported an experiment in which participants were simply asked to plot a single pair of points on a piece of graph paper, with the coordinates provided by the experimenter specifying a pair of points that lay at one of three different distances (close, intermediate, or far, relative to the range available on the graph paper). The participants who had graphed a more distant pair reported themselves as being significantly less close to members of their own family than did those who had plotted a more closely-situated pair. In another experiment, people's estimates of the caloric content of different foods were reportedly altered by the same type of spatial distance priming. Direct replications of both results were attempted, with precautions to ensure that the experimenter did not know what condition the participant was assigned to. The results showed no hint of the priming effects reported by Williams and Bargh (2008).



Priming: Replication studies

Two Failures to Replicate High-Performance-Goal Priming Effects

Christine R. Harris , Noriko Coburn, Doug Rohrer, Harold Pashler

Published: August 16, 2013 • https://doi.org/10.1371/journal.pone.0072467

Article	Authors	Metrics	Comments	Media Coverage	
*					

Abstract

Abstract Introduction

Experiment 1

Experiment 2

General Discussion

Conclusion

Author Contributions

References

Reader Comments (0)

Media Coverage (0)

Bargh et al. (2001) reported two experiments in which people were exposed to words related to achievement (e.g., strive, attain) or to neutral words, and then performed a demanding cognitive task. Performance on the task was enhanced after exposure to the achievement related words. Bargh and colleagues concluded that better performance was due to the achievement words having activated a "high-performance goal". Because the paper has been cited well over 1100 times, an attempt to replicate its findings would seem warranted. Two direct replication attempts were performed. Results from the first experiment (n = 98) found no effect of priming, and the means were in the opposite direction from those reported by Bargh and colleagues. The second experiment followed up on the observation by Bargh et al. (2001) that high-performance-goal oriming was enhanced by a 5-minute delay between priming and test. Adding such a delay, we still found no evidence for high-performance-goal priming in = 66). These failures to replicate, along with other recent results, suggest that the literature on goal priming requires some skeptical scrutiny.



Preclinical research (Freeedman et al. 2015)

The Economics of Reproducibility in Preclinical Research

Leonard P. Freedman , Iain M. Cockburn, Timothy S. Simcoe

Published: June 9, 2015 • https://doi.org/10.1371/journal.pbio.1002165

Article	Authors	Metrics	Comments	Media Coverage
*				

Correction

Abstract

Introduction

Defining Reproducibility

Analysis of Four Categories of Irreproducibility

Economic Impact of Irreproducibility

The Role of Best Practices and Standards

Conclusions

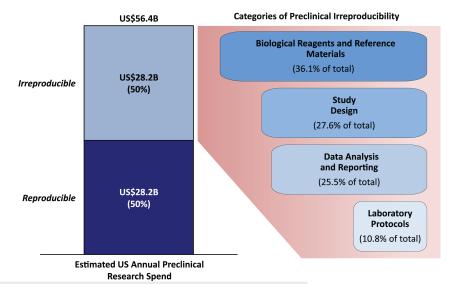
10 Apr 2018: The PLOS Biology Staff (2018) Correction: The Economics of Reproducibility in Preclinical Research. PLOS Biology 16(4): e1002626. https://doi.org/10.1371/journal.pbio.1002626 | View correction

Abstract

Low reproducibility rates within life science research undermine cumulative knowledge production and contribute to both delays and costs of therapeutic drug development. An analysis of past studies indicates that the cumulative (total) prevalence of irreproducible preclinical research exceeds 50% resulting in approximately US\$28,000,000,000 (US\$28B)/year spent on preclinical research that is not reproducible—in the United States alone. We outline a framework for solutions and a plan for long-term improvements in reproducibility rates that will help to

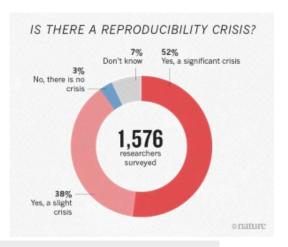


Preclinical research (Freeedman et al. 2015)



1,500 scientists lift the lid on reproducibility, Nature, 2016

Survey of 1,576 researchers who took a brief online questionnaire on reproducibility in research:



1,500 scientists lift the lid on reproducibility, Nature, 2016

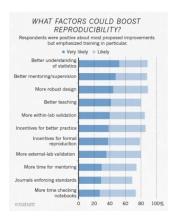
Survey of 1,576 researchers who took a brief online questionnaire on reproducibility in research:

- More than 70% of researchers have tried and failed to reproduce another scientist's experiments,
- More than half have failed to reproduce their own experiments
- pressure to publish and selective reporting always or often contributed [to reproducibilty issues]. > 50% pointed insufficient replication in the lab, poor oversight or low statistical power.



1,500 scientists lift the lid on reproducibility, Nature, 2016

Survey of 1,576 researchers who took a brief online questionnaire on reproducibility in research:



Recent example

⇒Lack of statistical power

SCIENCE

A Waste of 1,000 Research Papers

Decades of early research on the genetics of depression were built on nonexistent foundations. How did that happen?

ED YONG MAY 17, 2019



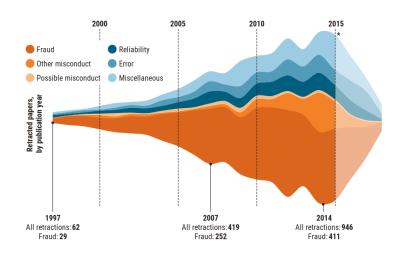


Recent example

"A waste of 1,000 research papers", the Atlantic, 2019:

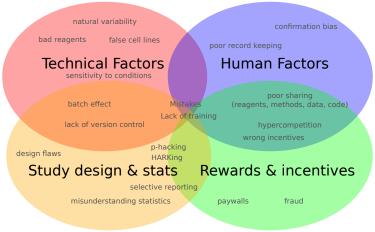
"What bothers me isn't just that people said [the gene] mattered and it didn't", wrote the pseudonymous blogger Scott Alexander in a widely shared post. "It's that we built whole imaginary edifices on top of this idea of [it] mattering". Researchers studied how SLC6A4 affects emotion centers in the brain, how its influence varies in different countries and demographics, and how it interacts with other genes. It's as if they'd been "describing the life cycle of unicorns, what unicorns eat, all the different subspecies of unicorn, which cuts of unicorn meat are tastiest, and a blow-by-blow account of a wrestling match between unicorns and Bigfoot", Alexander wrote.

What about retracted papers?



2.2 Initiatives

Factors decreasing reproducibility





from https://www.repro4everyone.org



2.2 Initiatives

Quote

"This focus on positive results is arguably one of the central drivers of the reproducibility crisis", Russel A. Poldrack, "The Costs of Reproducibility", Neuron, 2019"



2.2 Initiatives

A few pointers

- The Costs of Reproducibility, Neuron, 2019
- What does research reproducibility mean? Science Trans. Med., 2016
- The Economics of Reproducibility in Preclinical Research, Plos. Biol., 2015
- Community-led reproducibility workshops https://www.repro4everyone.org/
- 1,500 scientists lift the lid on reproducibility, Nature, 2016
- Leading individuals and institutions in adopting open practices to improve research rigour http://bulliedintobadscience.org/



2.3 Some definitions

Experimental variability

In experimental sciences, variablility of the results is mainly due to:

- Biological variations
 - Random nature of measured phenomena;
 - Different subjects, organisms, samples.
- Technical variations
 - Small changes in experimental conditions;
 - Noise of measurement tool;
 - Sample preparation.
- Same interpretation?

Even with all things equal otherwise



Computational variability

In data analysis: computers and programs are supposed to be exact!

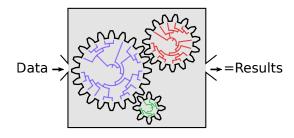
- ⇒ perfect reproducibility? Actually: No
 - Different versions of operating system;
 - Different versions of tools used;
 - Different hardware;
 - Random nature of some algorithms (simulations, etc.);
 - Numerical instability;
 - Parallel algorithms;
 - Poor method description;
 - etc.



Computational reproducibility

We can define several levels of reproducibility (Cohen-Boulakia et al., FGCS, 2017):

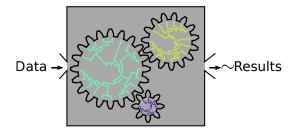
Repeat: The data analysis experiment is performed in the exact same computational setting as the original experiment. In that case, results should be exactly the same without any variation. This necessitate to gather as many information as possible about the initial experiment, i.e. all tools versions, all operating system library versions, the state of the random number generator, etc.;



Computational reproducibility

We can define several levels of reproducibility (Cohen-Boulakia et al., FGCS, 2017):

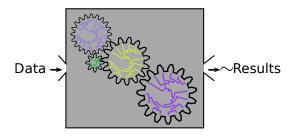
Replicate: The data analysis experiment can be performed in a slightly different environment (different tool versions, different library versions, different random seeds, etc.), but the general protocol remains the same. In that case, results are not exactly the same, but scientific interpretation should be identical;



Computational reproducibility

We can define several levels of reproducibility (Cohen-Boulakia et al., FGCS, 2017):

Reproduce: The data analysis experiment aims at validating the scientific hypothesis, and can be performed in a different environment and with a different protocol (different tools, different workflow, etc.). This level of reproducibility gives us the best level of confidence about the quality of the results.



2.5 An so now...

Summary

Technical difficulties and some solutions to improve computational reproducibility:

- Technical difficulties with usual practices
- Solutions:
 - Data management
 - Software/Script development
 - Environment management
 - Analysis development (workflows, notebooks, etc.)

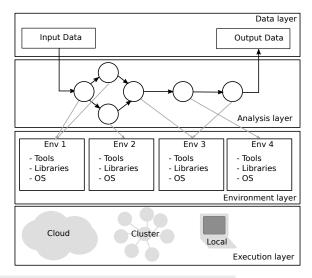


Difficulties and solutions



3.1 Whole analysis

Analysis stack

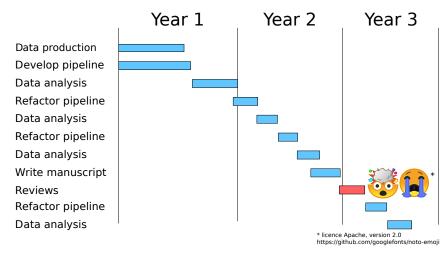


Data management: Difficulties

new_analysis_latest	4,1 ko do	ssier		Aujourd'hui	
new_analysis_v1	4,1 ko do	dossier		Aujourd'hui	
new_analysis_v2	4,1 ko do	ssier		Aujourd'hui	
prev_analysis_v1	4,1 ko do	4,1 ko dossier		Aujourd'hui	
prev_analysis_v2	4,1 ko dossier		Aujourd'hui		
New_data		4,1 ko	dossier		Aujourd'h
New_results		4,1 ko	dossier		Aujourd'hu
Old_results		4,1 ko	dossier		Aujourd'hu
Results 2019 08		4.4 1	dossier		Aujourd'hu

Data management: Difficulties

Typical process:



Data management: Difficulties

After Review:

- How was this figure generated?
- Where is the right data version???
- Where is the right script version???
- How was this file called???



Data management: Some solutions

Directory structure

```
Project name
            Raw Data
                     README
            Methods
            Results
                    Results method 1
                              2019...
            Scripts
            Manuscript
                   -Version date
            README
```

Inspired from https://www.repro4everyone.org

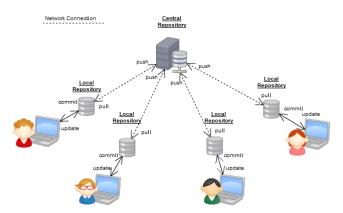


Data management: Some solutions

- File naming convention (from https://www.repro4everyone.org, CC BY)
 - Example: Date_Project_Experiment_Type_ID_Version.xlsx
- Versioning of scripts, manuscripts, etc.: Why not Git?



What is a Distributed Version Control System (VCS)?

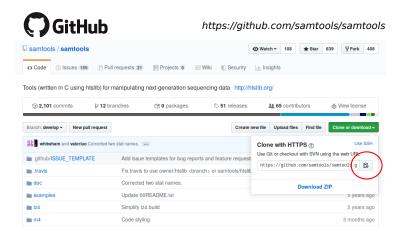


from http://www.tranthanhtu.vn/post/2017/01/11/git-branching-model

 $Slide \ from \ C3BI \ Tutorials \ https://github.com/C3BI-pasteur-fr/tutorials$



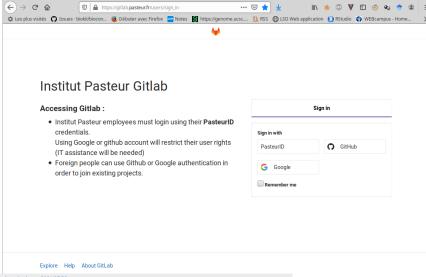
Git Basics: Clone a github repository



\$ git clone https://github.com/samtools/samtools.git



Git at Pasteur: GitLab (gitlab.pasteur.fr: ∼ local GitHub)





Data management: Take home message

- Project Structure
- Versionning
- Storage/Backup



3.3 Software/Script development

Difficulties

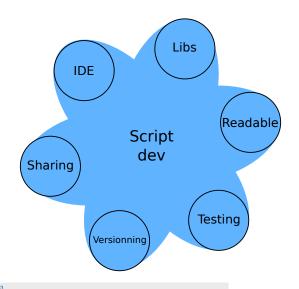
- Developing good code
- Do not reinventing the wheel
- Writing understandable code
- Do not introducing (too much) bugs
- Maintaining/Sharing the code
- Versioning the code (again)
- Executing, Deploying (dependencies, etc.)

Your software/script has at least ONE user: the future you, think about them!



3.3 Software/Script development

Coding: Good practices



What is the computational environment?

The computational environment is made of:

- The operating system:
- The core libraries: Versions!
- The tools used: Versions and OS!
- Their dependencies: Versions and OS!

Difficulties

- LOTS of Informations needed to capture and describe the whole environment!
- Different tools may have conflicting dependencies...
- Keep old tools to rerun old analyses...
- Manage a huge tool repository...



Computational environment: solutions

Several solutions:

- Virtual machines
- Conda / Bioconda
- Docker
- Singularity
- etc.



Computational environment: Virtual Machines (VMs)

Virtual Machine
Application
Guest OS
Virtual File System
Hypervisor
Host OS
Hardware



- Pros
 - Everything is included
 - No dependency problem
- cons
 - VMs include their own OS
 - VMs consume lots of resources
 - VMs are heavy: large storage
- Not ideal in a context of data analysis



Computational environment: Conda/Bioconda

Conda: package and environment management system (https://docs.conda.io/en/latest/miniconda.html).



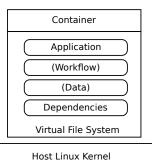
- pros
 - Create several environments with their own tools and dependencies (VERY useful)
 - Keep as many environments as the number of old analyses
- cons
 - Still OS specific: tools may behave differently in MacOS and Linux...
 - Difficult to freeze all dependency versions



Computational environment: Containers



Docker and Singularity allow to package applications/tools and their dependencies in an isolated "Container" that can be executed on any server.



- Lightweight! (dozens of MB for main single application containers)
- Fast and efficient: No need to boot a full OS
- Can keep all containers (very old anlyses, etc.)

Next: Analysis development

We know how to:

- Manage data
- Develop independent analysis scripts
- Manage environment (tool versions, etc.)

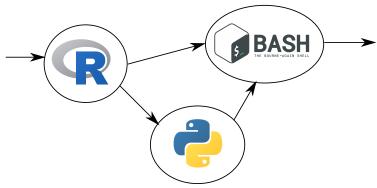
But...

How to link all of them together in a full analysis pipeline?



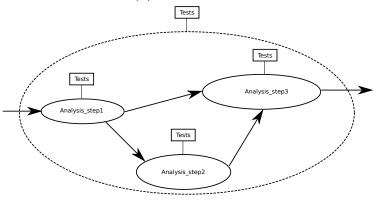
Analysis development: Difficulties

Multi-language: R, python, bash, awk, etc.



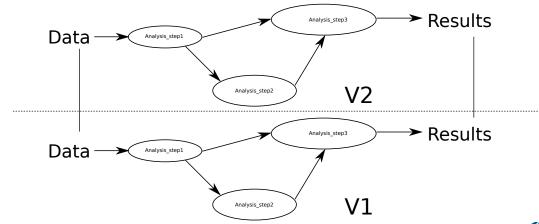
Analysis development: Difficulties

Testing: How to test the whole pipeline?



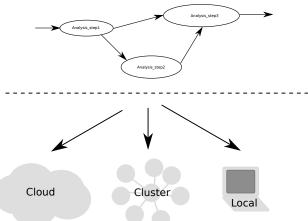
Analysis development: Difficulties

Versionning



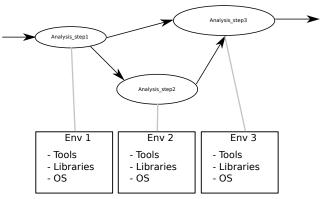
Analysis development: Difficulties

→ Different execution machines: local, cluster, cloud, etc.



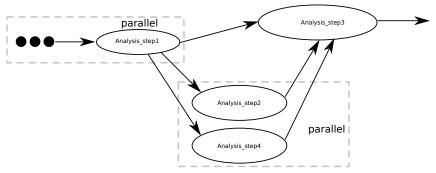
Analysis development: Difficulties

High dependency to the environment (cluster scheduler, tool versions, etc.)



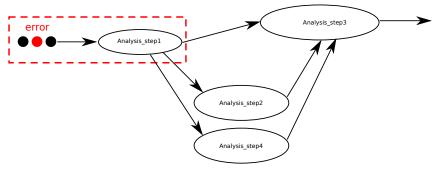
Analysis development: Difficulties

Parallel processing



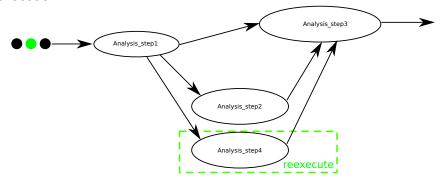
Analysis development: Difficulties

Error handling



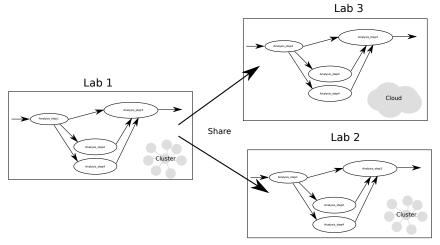
Analysis development: Difficulties

Re-execution



Analysis development: Difficulties

Maintenance, sharing, reuse, reproduce



Analysis development: Solutions

Notebooks (Jupyter, RMarkdown)



Workflow systems (Nextflow, Snakemake)





Analysis development: Notebooks

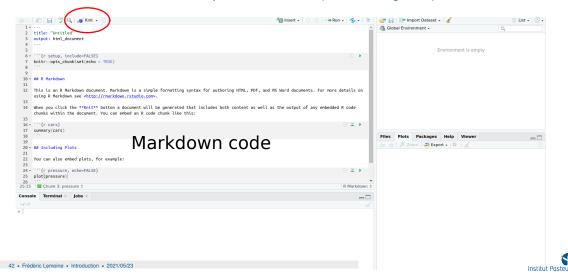
Interactive notebooks are a relatively new analysis technology which allows highly interactive data exploration, visualizations, exhaustive documentation, and sharing of an analysis process. Associated with the concept of Literate Programming, these notebooks are valuable tools towards better analyses comprehension and reproducibility. There are many notebooks solutions to choose from:

- Jupyter: https://jupyter.org/
- R Markdown: https://rmarkdown.rstudio.com/
- Apache zeppelin: https://zeppelin.apache.org/
- **Google Colaboratory**: https://colab.research.google.com
- Observable (client-side): https://observablehq.com/
- Spark notebooks: http://spark-notebook.io/
- Beaker (engulfed by Jupyter)
- And more ...



Analysis development: RMarkdown

Mix R code, comments, and report in a text file (Versionning, etc.):



Analysis development: RMarkdown

Nice rendering (report or slides):

Untitled

R Markdown

This is an R Markdown document. Markdown is a simple formatting syntax for authoring HTML, PDF, and MS Word documents. For more details on using R Markdown see http://rmarkdown.rstudio.com.

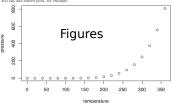
When you click the Knit button a document will be generated that includes both content as well as the output of any embedded Rogole chanks within the document. You can embed an R code chank like this:

summary(cars)



Including Plots

You can also embed plots, for example:



Note that the echo = FALSE parameter was added to the code chunk to prevent printing of the R code that generated the plot.



Analysis development: Jupyter notebooks

Mix several languages, comments, and report in a unique notebook, with live execution:

- extensibility (numerous plugins)
- recognition and pretty formatting by GitLab and GitHub
- helpful community

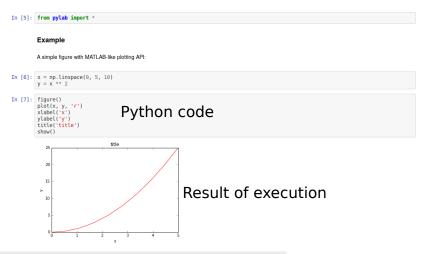
Getting started (with conda!):

```
conda create -n jupyter jupyter jupyterlab
conda activate jupyter
jupyter lab
```



Analysis development: Jupyter notebooks

Nice interactive rendering:



Analysis development: Notebooks downsides

- They can be cumbersome (cell order execution...) for development and not necessarily a good first entry point in Programming
- They do not actively help in developing good coding practices (no proper module/library design)
- Source control difficulties
- Testing difficulties
- Not easy to use on different clusters...
- Not easy to use several environments (tool versions, etc.)



Analysis development: Workflow systems

In the past 20 years, bioinformatics workflow systems have been developed to solve lots of the difficulties described earlier. They allow to:

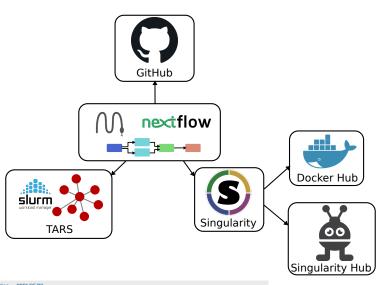
- Structure and develop their analysis pipelines
- Automate and monitor their execution
- Trace data flow and results
- Abstract from the execution machines and environment

Most popular options:

- Galaxy: https://usegalaxy.org/
- SnakeMake: https://snakemake.readthedocs.io/en/stable/
- Nextflow: https://nextflow.io



Analysis development: Workflow systems





3.6 Summary

Full stack

