Reproducibility for everyone

https://tinyurl.com/plantbio-repo

addgene
The nonprofit plasmid repository

eLIFE

CODE OCEAN BETA

protocols.io

Why does reproducibility matter to you?

addgene
The nonprofit plasmid repository

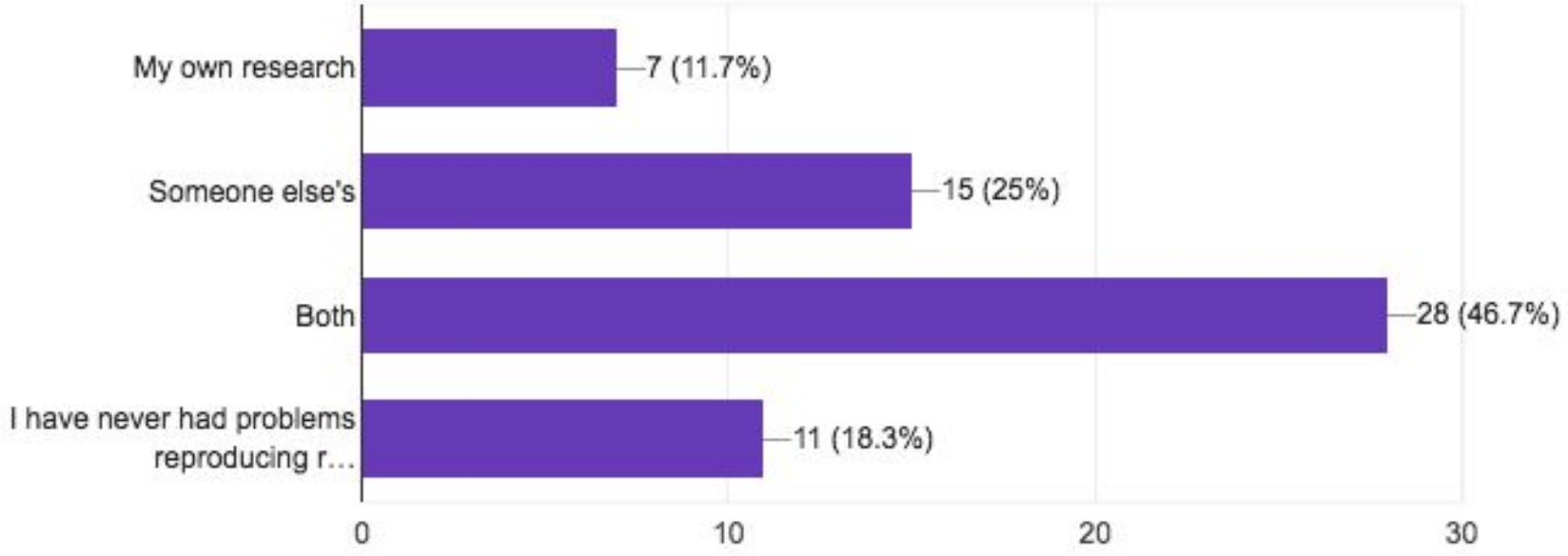
eLIFE

CODE OCEAN BETA

protocols.io

Have you ever had problems reproducing your own or someone else's research?

60 responses



Goals and objectives

- ‘Reproducibility’ framework
- ‘Reproducibility’ tools
- Starting point of a ‘lifelong’ journey

Introduction

- What does reproducibility mean?
- What are the different modes of reproducibility?
- Is reproducibility all that matters?

- ‘Reproducibility’ tool shed.
    - organization
    - documentation
    - analysis
    - dissemination

What does reproducibility mean?

What does reproducibility mean?

Reproducible research: Authors provide all the necessary data and the computer codes to run the analysis again, re-creating the results.

Replication: A study that arrives at the same scientific findings as another study, collecting new data and completing new analyses.

Barba, 2018
https://arxiv.org/abs/1802.03311

Schloss, 2018
10.1128/mBio.00525-18

What are the different modes of ‘reproducibility’?

Methods	Same experimental system	Different experimental system
Same methods	Reproducibility	Replicability
Different methods	Robustness	Generalizability

Schloss, 2018
10.1128/mBio.00525-18

Introduction

What are the different modes of ‘reproducibility’?

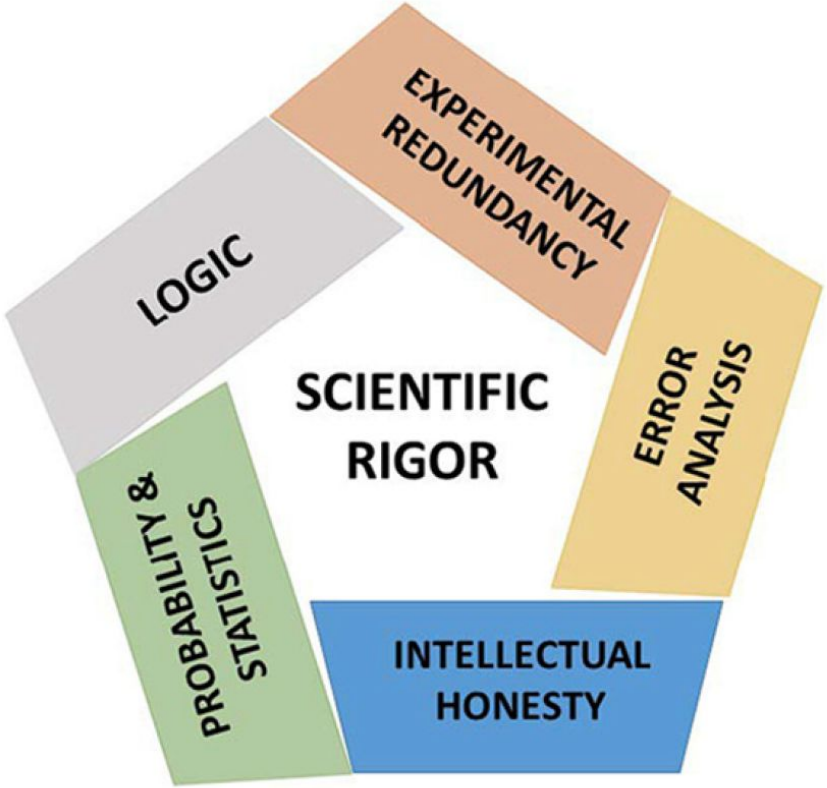
Methods	Same experimental system	Different experimental system
Same methods	Reproducibility	Replicability
Different methods	Robustness	Generalizability

Reproducibility is the minimum standard for science.

Schloss, 2018
10.1128/mBio.00525-18

Is reproducibility all that matters?

Introduction

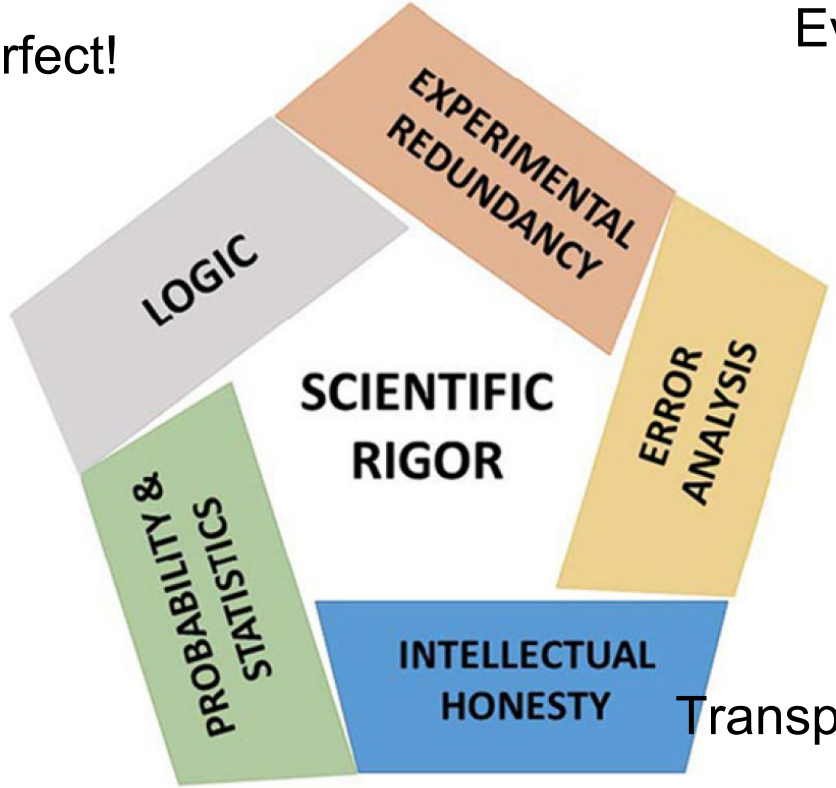


Casadevall and Fang, 2016
10.1128/mBio.01902-16

Introduction

No one is perfect!

Every little helps!



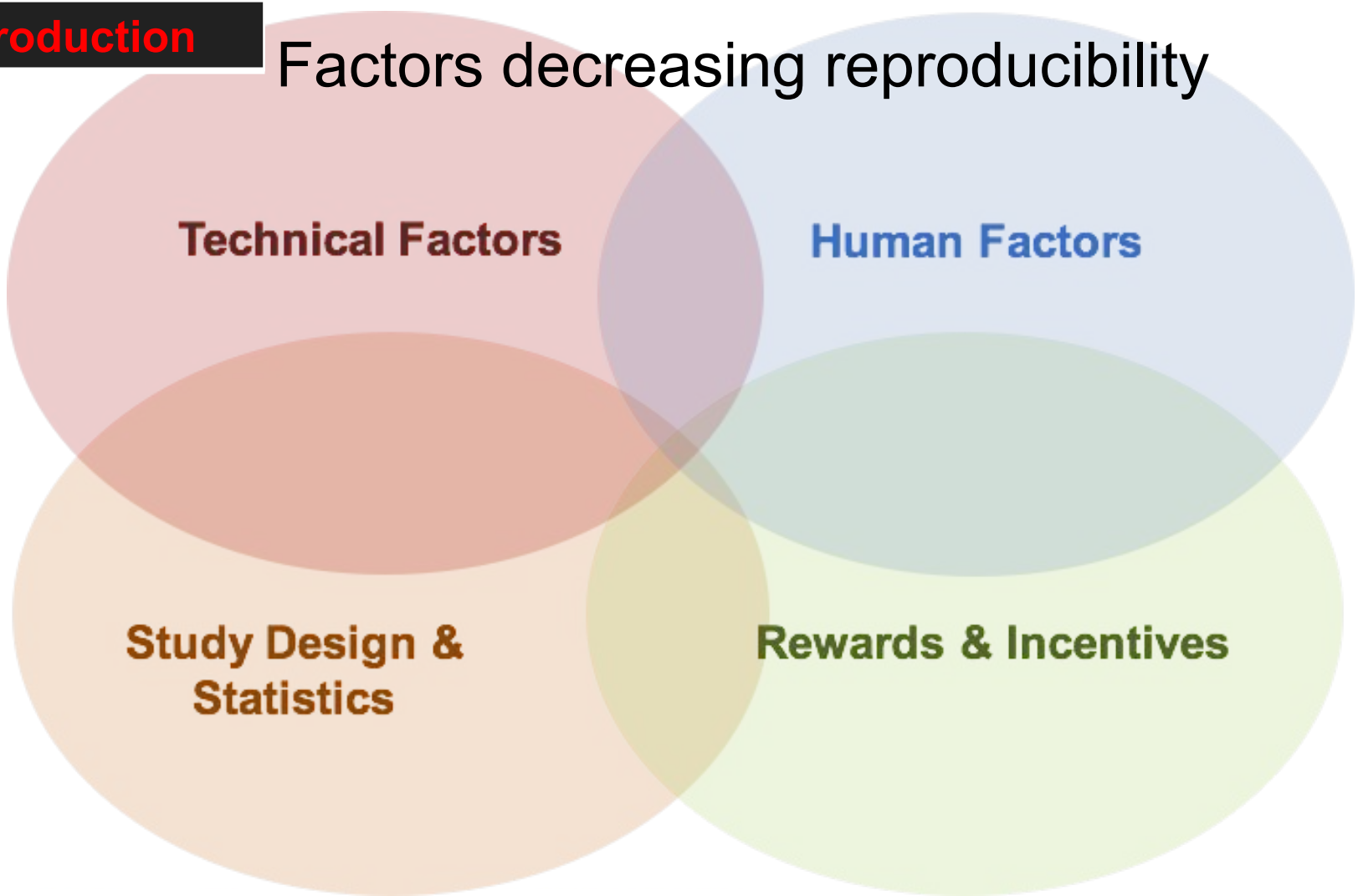
Transparent and open science!

Everyone starts somewhere!

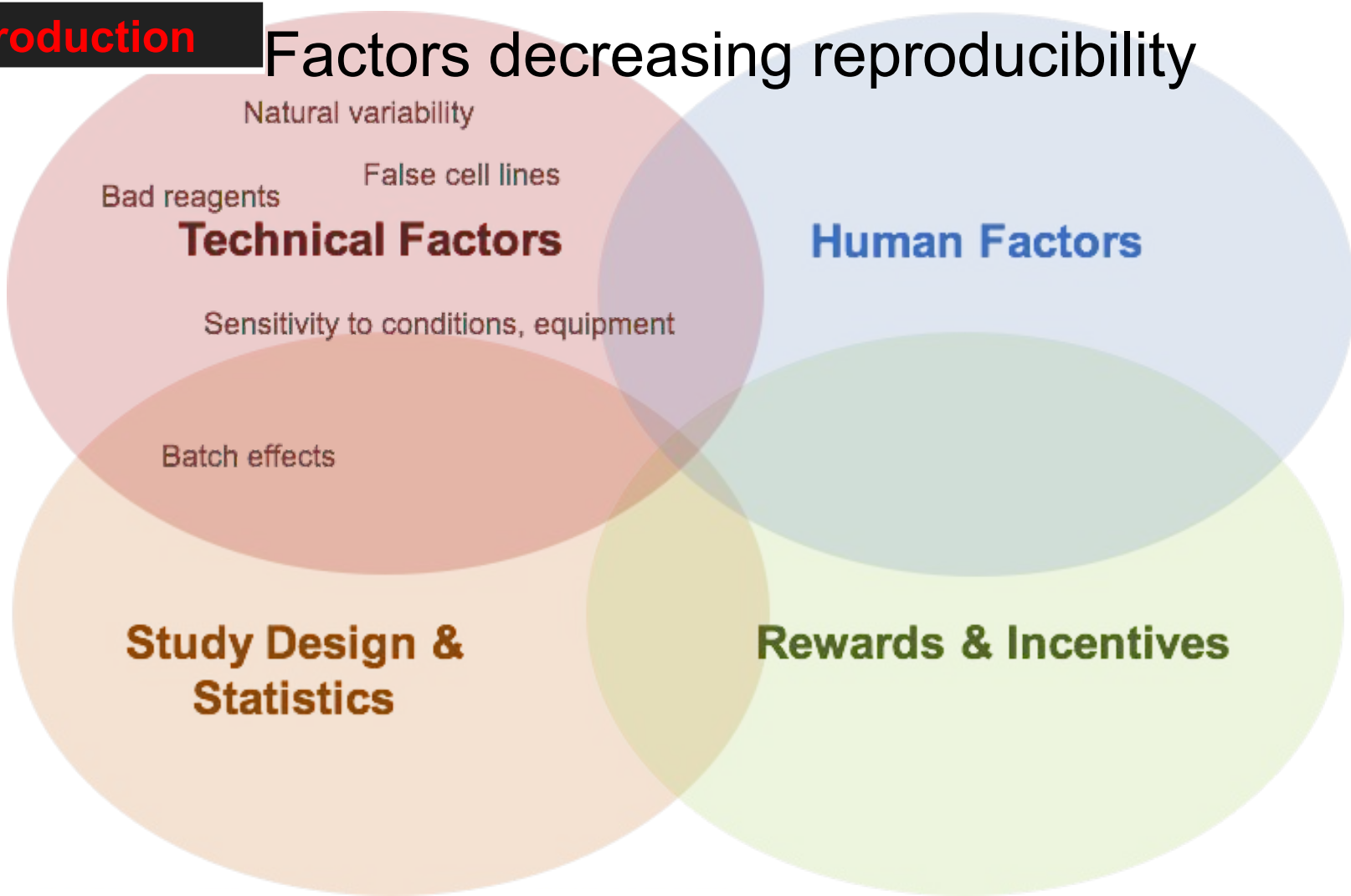
Casadevall and Fang, 2016
10.1128/mBio.01902-16

Factors decreasing reproducibility

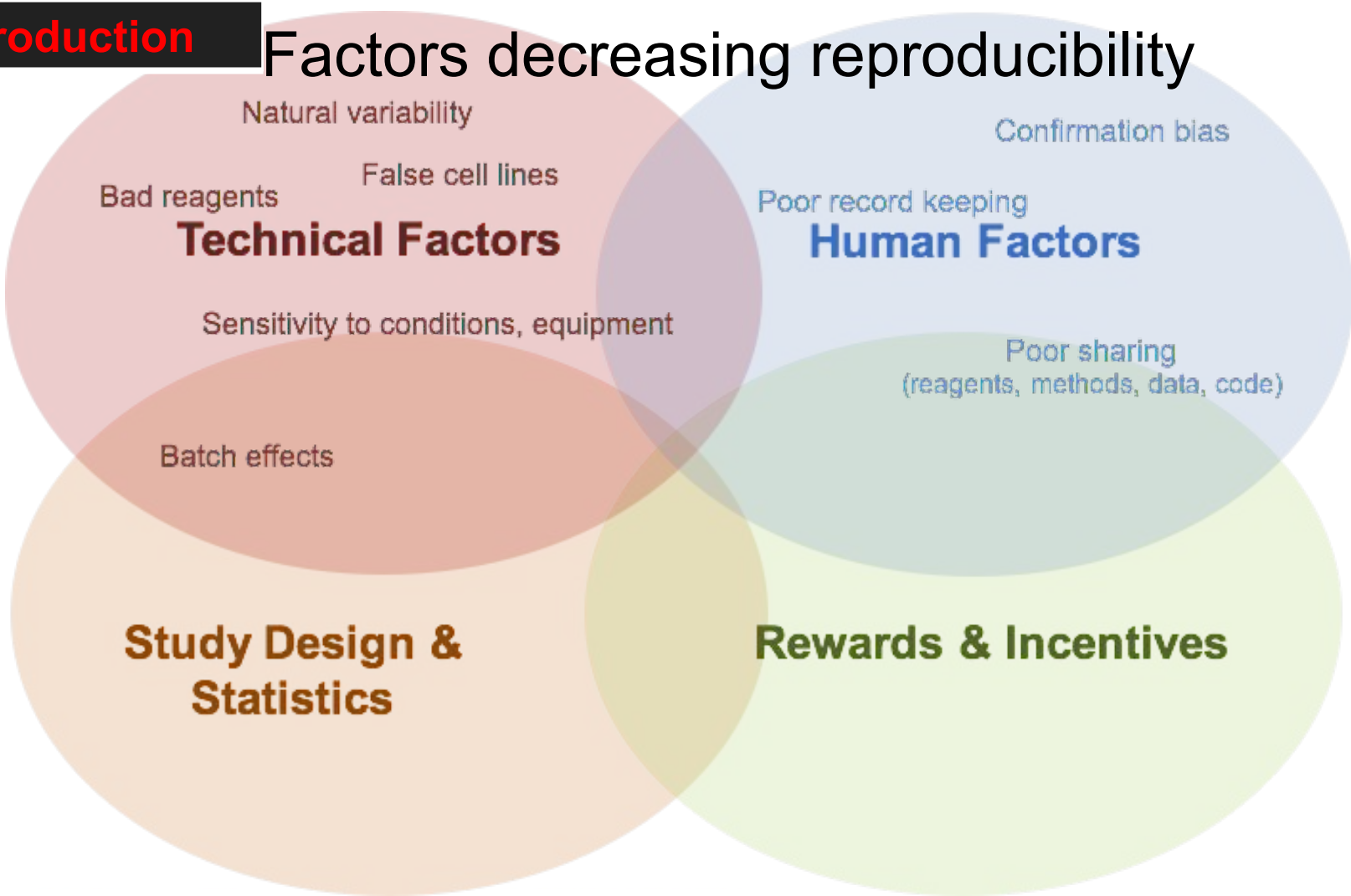
Factors decreasing reproducibility



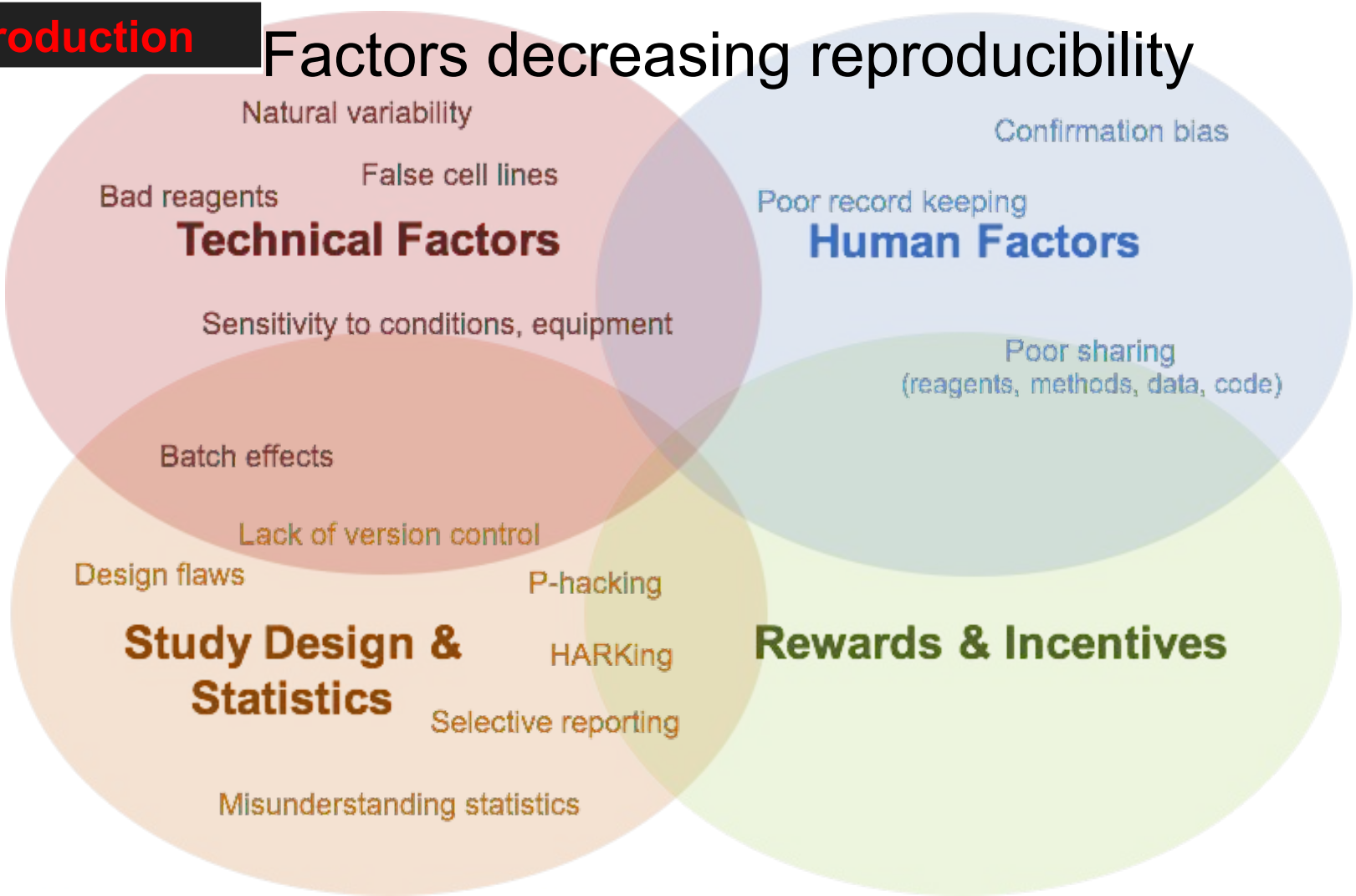
Factors decreasing reproducibility



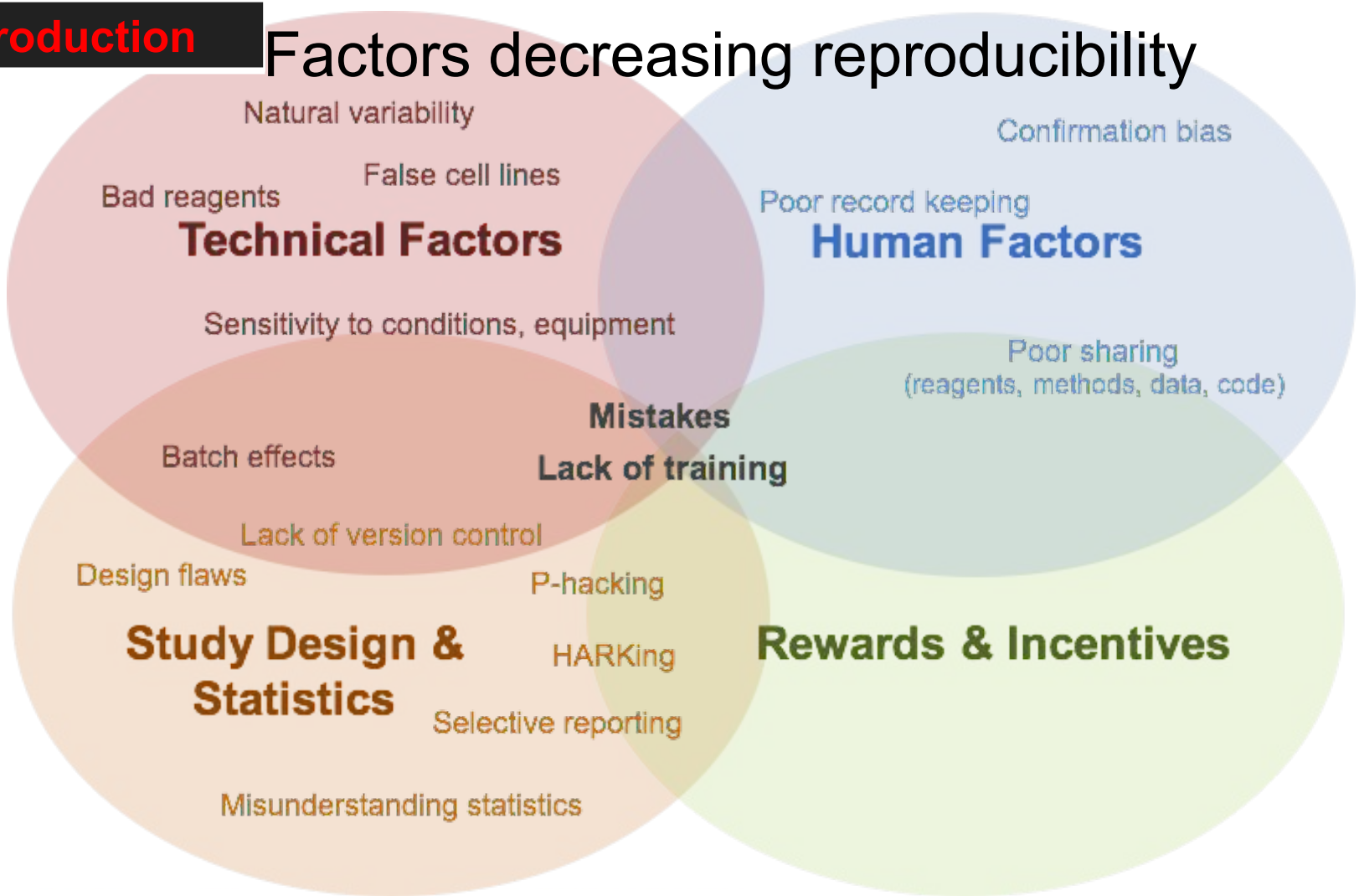
Factors decreasing reproducibility



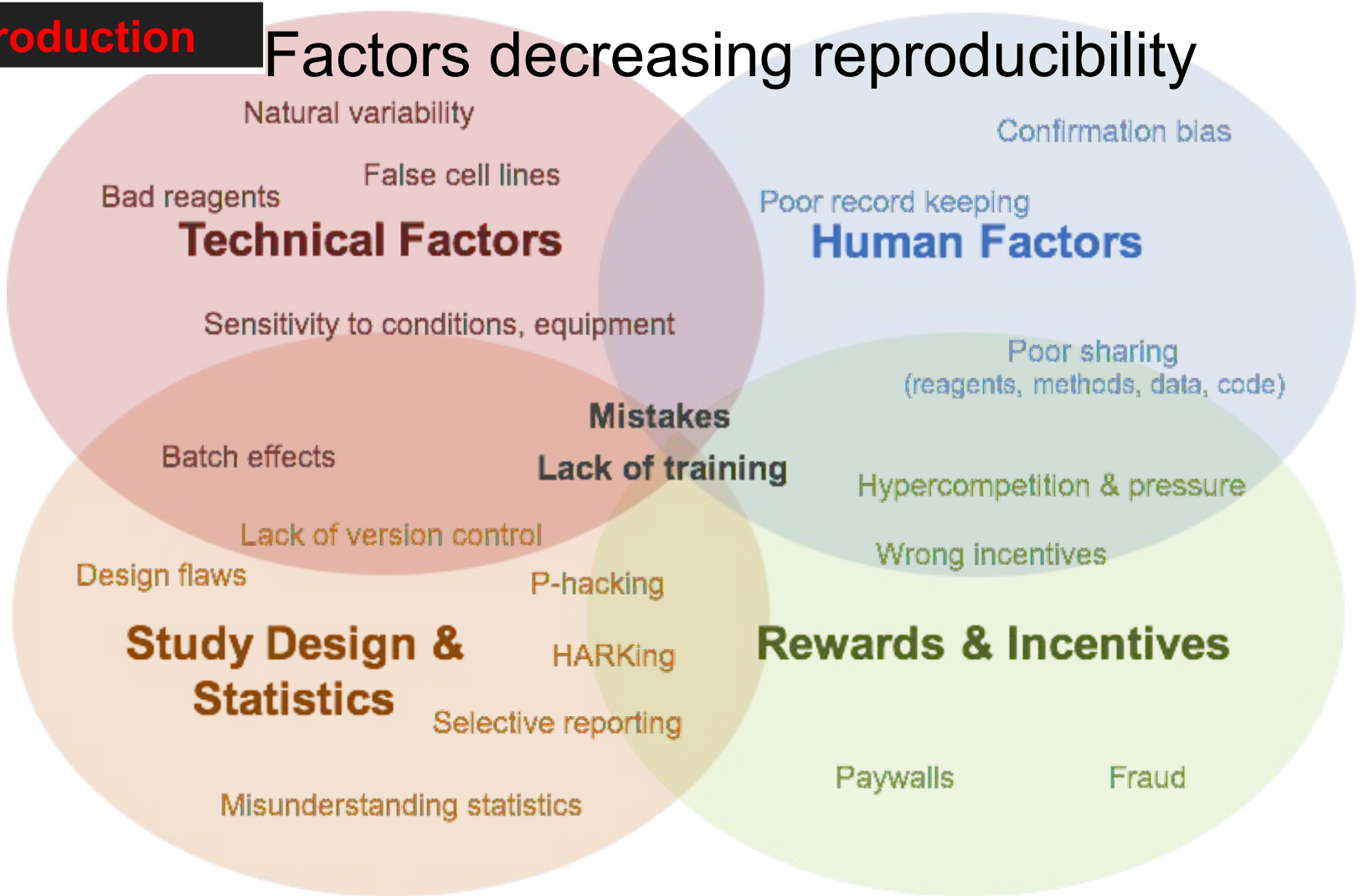
Factors decreasing reproducibility



Factors decreasing reproducibility



Factors decreasing reproducibility



There is good news!

Richard Harris,
NPR science journalist

Author of “Rigor Mortis” (book on biomed reproducibility)

“So what’s a careful scientist to do? First and foremost, be aware of the conditions around you that may increase the risk of irreproducible results, whether they are bad ingredients, dubious statistical traditions, or outside pressures that can shape behavior. **Also take heart. This reproducibility “crisis” isn’t really a crisis at all**. These are not new problems. Rather, I think of this moment as an awakening. And that’s a good thing, because we need to recognize that a problem exists before we can seek solutions.”

https://cen.acs.org/articles/95/i47/Reproducibility-issues.html

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What can we do by the end of the century?

Cori Bargmann, HHMI investigator, President CZI Science

"82 years ago, there were no antibiotics & we didn't know that smoking causes lung cancer... We can expect a lot from the next 82 years."

What can we do by the end of the century?

Cori Bargmann, HHMI investigator, President CZI Science

"82 years ago, there were no antibiotics & we didn't know that smoking causes lung cancer... We can expect a lot from the next 82 years."

"**Where can we be in 82 years if we accelerate science?**"

Where is your greatest potential for growth?

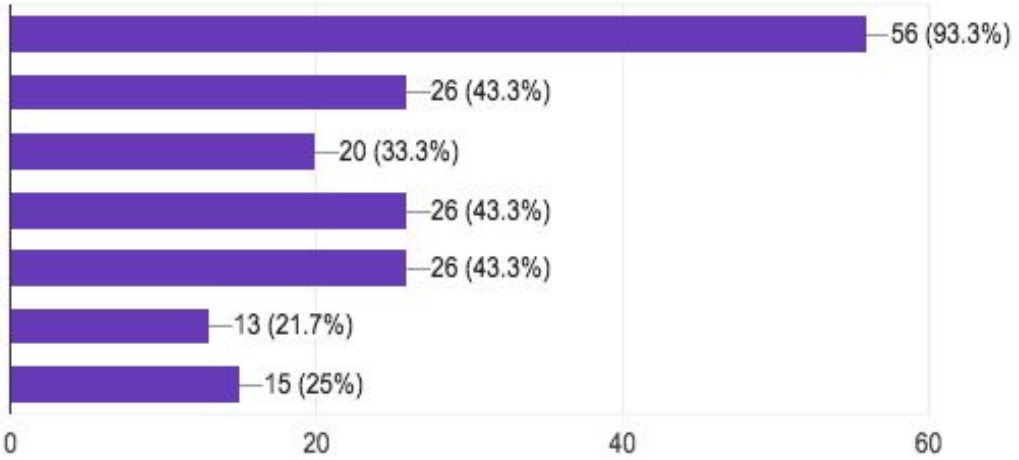
Where is your greatest potential for growth?

More detailed methods, analysis and record keeping

More publicly available data including meta-data

Fewer incentives to be first rather than right

Better reagent sharing e.g. plasmids, antibodies ...



Data management

http://kbroman.org/dataorg/

https://dmptool.org/

organization	documentation	analysis	dissemination

I cannot find this file!

What did I call that file again?

Where is my file?

Was this the wild type picture or the mutant one?

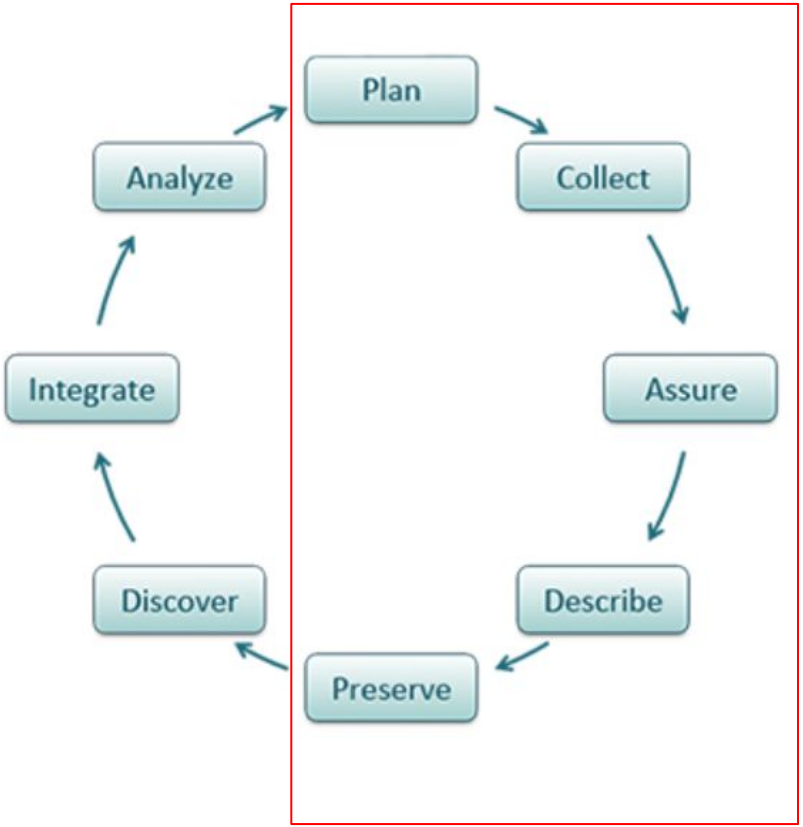
What version was it?

Where is my RAW!!! data?

Have a plan! Be happy!

Think about….

- **What** data will be produced as a part of the project
- **How** each type of data will be organized, documented, standardized, stored, protected, shared and archived
- **Who** will take responsibility for carrying out the activities listed above, and
- **When** these activities will take place over the course of the project (and beyond)
- **Metadata**



http://guides.lib.purdue.edu/c.php?g=353013&p=2378292

https://www.dataone.org/best-practices

Project directory structure

Project_1

- methods
- raw_data
- analysis
- scripts
- manuscript
- readme and/or ELN link

Inspired by ‘Bioinformatic data skills’ Vincent Buffalo

Project directory structure

```
Project_1
    methods
    raw_data
        readme
    analysis
        analysis_method_1
            2017
            2018
        analysis_method_2
    scripts
    manuscript
        text
            version_1
    readme and/or ELN link
```


Inspired by ‘Bioinformatic data skills’ Vincent Buffalo

Project directory structure

- Project_1
  - methods
  - raw_data
    - readme
  - analysis
    - analysis_method_1
      - 2017
      - 2018
    - analysis_method_2
  - scripts
  - manuscript
    - text
      - version_1
  - readme and/or ELN link

Always keep **raw** data!

Always backup your data! X 3

Inspired by ‘Bioinformatic data skills’ Vincent Buffalo

Project directory structure

- Project_1
  - methods
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    - text
      - version_1
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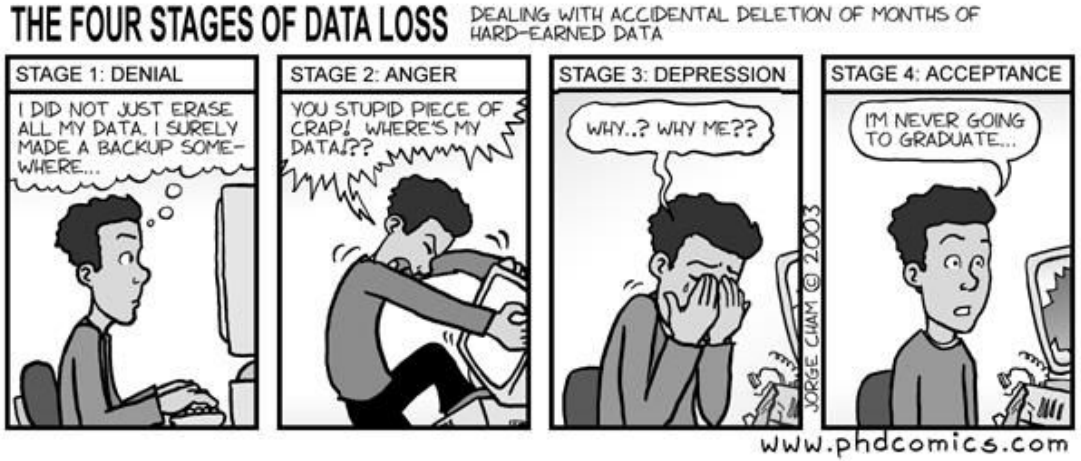
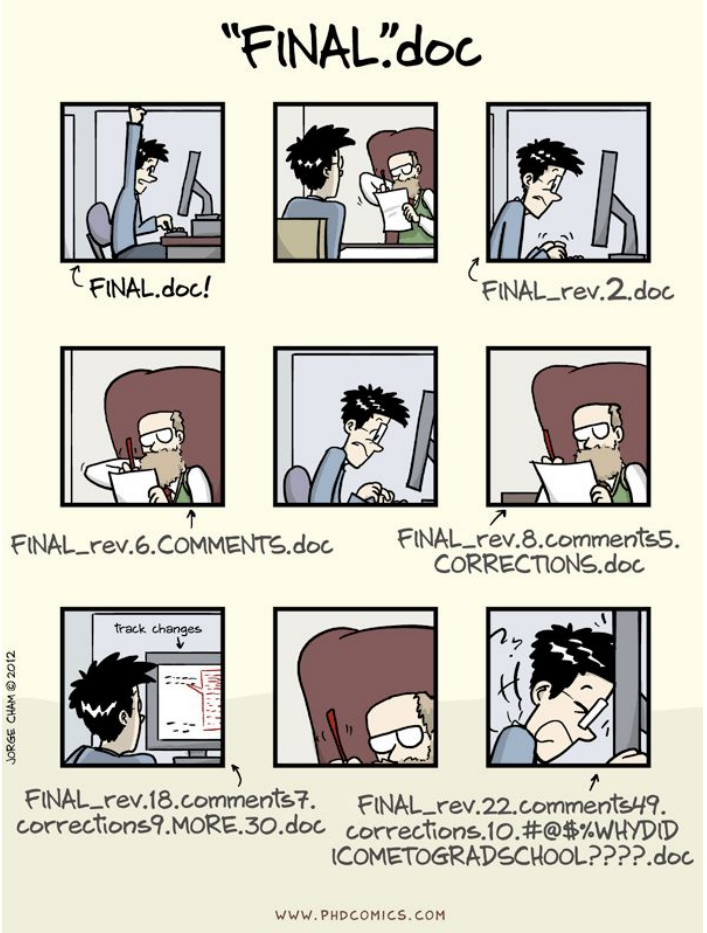
Always keep **raw** data!



Inspired by 'Bioinformatic data skills' Vincent Buffalo

How did you call the last file you generated?

Did you have a plan?

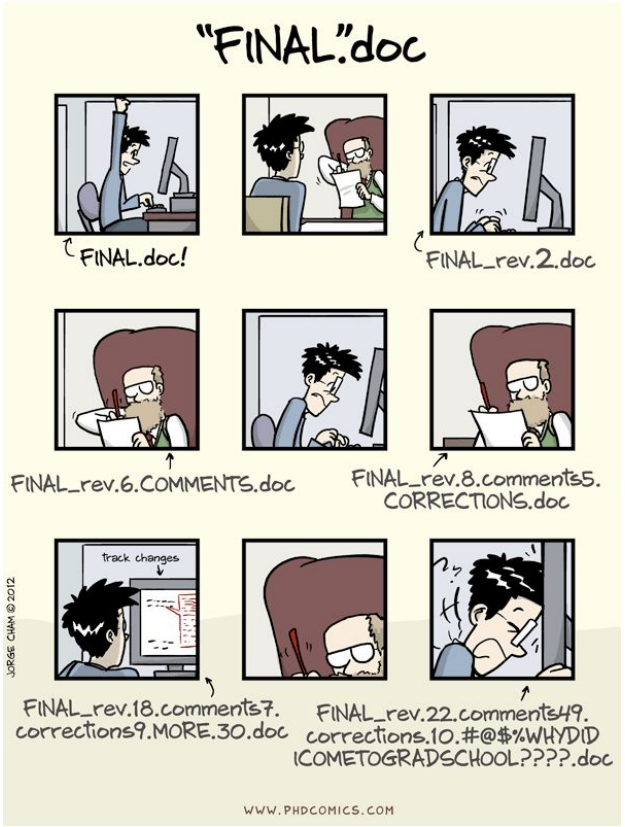


http://guides.lib.purdue.edu/c.php?g=353013&p=2378292

http://kbroman.org/dataorg/

File naming convention (FNC)

- Test_data_2013
- Project_Data
- Design for project.doc
- Lab_work_Eric
- Second_test
- Meeting Notes Oct 23



http://guides.lib.purdue.edu/c.php?g=353013&p=2378292

http://kbroman.org/dataorg/

File naming convention (FNC)

- Include date in yyyy-mm-dd format
- Use meaningful abbreviations
- Have group identifiers
- Document your decisions
- Be consistent

http://guides.lib.purdue.edu/c.php?g=353013&p=2378292

http://kbroman.org/dataorg/

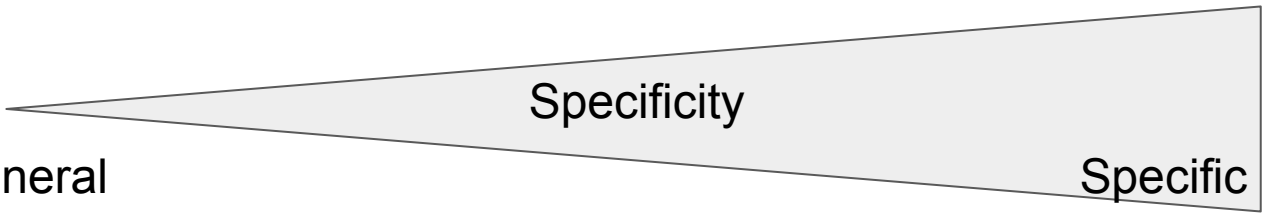
File naming convention (FNC)

- Include data in yyyy-mm-dd format
- Use meaningful abbreviations
- Have group identifiers
- Document your decisions
- Be consistent

20130825_DOEProject_Ex1Test1_Data_Gonzalez_v3-03.xlsx

Date **Project** **Type** **Version**

Experiment **ID**



http://guides.lib.purdue.edu/c.php?g=353013&p=2378292

- Findable
- Accessible
- Interoperable
- Reusable

Get organized! Be happy!

https://tinyurl.com/plantae-FAIR

Electronic Notebooks

Paper Lab-notebooks - in use since the 15th Century!

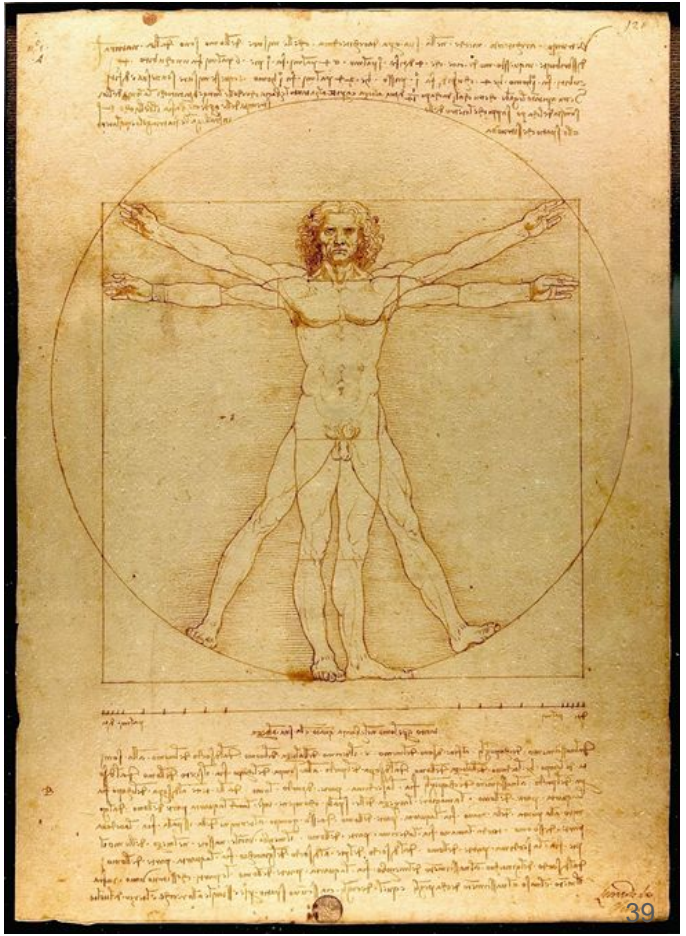
Good record keeping is important for

- Dissemination of ideas, findings
- Legally binding record that protects intellectual property

Not searchable!

Can be easily damaged, misplaced.
Not easy to back up.

Hard to share with collaborators.



Leonardo da Vinci's notebook, Codex Arundel c. 1458-1518 British Library

Why should you use an Electronic Lab Notebook?

Google docs

Store text electronically, Attach Images,
15 GB data limit, Multiple authors possible

Dropbox

Good for sharing data, 2 GB cloud storage limit (free version)

+ Many more Features!

Searchable

Export data as PDF (must back-up data regularly)

Easily shareable

Embed high res images, protocols etc

Easily accessible world over

Use the mobile App to quickly upload images

Cost considerations - Softwares available

Paid for— Bio-Itech, LabArchives, LabGuru

Paid (with free version)— SciNote, Benchling

Open source— Open wet ware, ELOG

Free— OSF (Open Science Framework), LocalWiki

Kanza, 10.1186/s13321-017-0221-3

One-size does not fit all!

Parameters to consider

Available lab notebooks

Features	Specifications														
	Benchling	Biovia	Confluence	Docollab	ECL	ELOG	Evernote	Exemplar	Findings	Hivebench	IDBS	LabArchives	LabCollector	LabWare	LabVantage	LabW
Interactivity																
Intuitive Interface Design	✓	No response received	*	*	No response received	*	No response received	No response received	*	*	*	✓	No response received	*	*	*
Auto Metadata Harvest	*	No response received	✗	✓	No response received	✗	No response received	No response received	✗	✓	*	✓	No response received	✗	✓	*
Search functions can search across file formats and beyond typos	*	*	*	*	No response received	*	*	*	*	*	*	*	No response received	*	*	*
Ability to manipulate files and images	*	No response received	*	*	No response received	*	No response received	*	*	*	*	*	No response received	*	*	*
Support for multiple open windows	✓	*	✓	✓	No response received	✓	✓	*	*	✓	✓	✓	No response received	✓	*	*
Ability to link out	✗	No response received	*	✓	✓	✓	✓	✓	✓	✓	✓	✓	No response received	✓	✓	✓
Support for Researcher Documentation																
Hyperlink support	✓	No response received	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	No response received	✓	✓	✓
Metadata Creation Prompts	✓	No response received	✗	✓	No response received	✓	✗	No response received	✗	✓	✓	✓	No response received	✗	✓	✓
Rights Management (licensing)	*	No response received	*	✓	No response received	*	No response received	No response received	✓	✓	*	✓	✓	✓	✓	✓
Protocol Integration	✓	*	✓	✓	No response received	✓	*	*	✓	✓	✓	✓	*	✓	*	✓
Adaptability to Lab workflows																
Accounts/Permissions Levels	✓	No response received	*	✓	✓	✓	✓	*	*	✓	✓	✓	✓	✓	*	✗
Internal Data Sharing	✓	*	*	✓	No response received	✓	✓	No response received	✓	✓	✓	✓	✓	✓	✓	*
Adaptable to a Variety of Workflows	*	No response received	*	*	No response received	*	No response received	*	*	*	*	*	*	*	*	*
Compatibility with authoring tools	✓	No response received	*	✓	No response received	✗	No response received	No response received	✗	*	*	✓	No response received	✗	*	✗
Windows Compatible	✓	No response received	✓	✓	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓
Macintosh Compatible	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	*
Linux Compatible	✓	✗	✓	✓	No response received	✓	No response received	✓	✗	✓	✓	✓	No response received	✓	✗	*
Android Compatible	✓	✓	✓	✓	No response received	✓	✓	✓	✗	✗	✓	✓	✓	✓	✓	✗
iOS Compatible	✓	✓	✓	✓	No response received	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗
Storage																
Cloud Storage	✓	No response received	✗	✓	No response received	✓	No response received	No response received	✓	✓	✓	✓	✓	✓	✓	✗
Local Storage	✗	No response received	✓	✗	No response received	✓	No response received	No response received	✓	✗	✓	✓	No response received	✓	✓	✓
Hybrid (cloud/local) Storage	✗	No response received	✗	✗	No response received	✗	No response received	No response received	✓	✓	✗	✓	No response received	✗	✗	✗
Versioning	*	*	*	*	No response received	*	No response received	*	*	*	*	*	*	*	*	*
File Redundancy	*	No response received	*	*	No response received	*	No response received	No response received	*	*	*	*	No response received	*	*	*
Creates stable URLs or persistent identifiers for entries	✓	No response received	✓	✓	No response received	✓	No response received	No response received	*	✓	✓	✓	No response received	✓	✓	✓
Can unregistered users access the data found at persistent links?	✓	No response received	✓	✗	No response received	✗	No response received	No response received	*	*	✗	✗	No response received	✗	✗	✗
Storage Capacity - Users	*	No response received	*	*	No response received	*	*	No response received	*	*	*	*	No response received	*	*	*
Storage Capacity - Max File Size	*	No response received	*	*	No response received	*	No response received	No response received	*	*	*	*	No response received	*	✓	*

ELN Features Matrix

https://datamanagement.hms.harvard.edu/electronic-lab-notebooks

Basic features of an Electronic Lab notebook

labarchives
Noble Research Institute edition

Data shared with authorized personnel eg: Supervisor

Search Notebook

Search by keyword, date

Notebook Navigator

A K Scheible

Inbox (1)

A K - Scheible

- Protocols
  - PCR-pET28, RALF 8/12
  - Arabidopsis
  - Medicago seeds
  - Making Solutions & Media
  - Primers
  - pET 28
  - pENTR cloning & Transformation
  - pET 28 Legin 17
  - Gen 5 Bioluminescence
  - Calcium Bursts-microscope
  - New...
- Experimental Data
- Ideas
- Lab Meeting Notes
- Presentation
- References
- Project 1
- Research Notes
- New...

Organize as needed

pET 28

Add Entry | Rich Text | Attachment | Office document | More

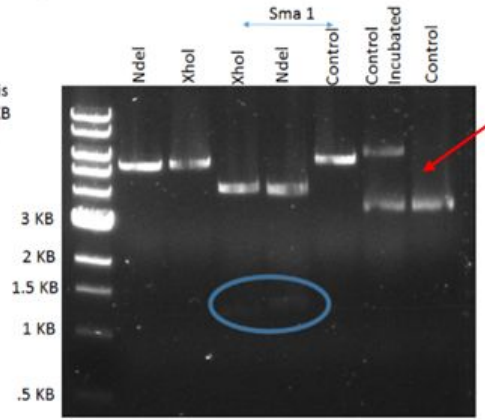
Office compatible

Page Tools

K A Jun 07, 2018 @08:36 AM CDT

Can print, share

pET 28 Digestion with NdeI, XhoI, and Sma 1



The Control Incubated is knicked at around 5.3 KB and has 1 specific cut.

Both of the pET Controls are supercoiled at 3.8 KB. The expected is 5.3 KB.

From the Sma Control and the Control Incubated we see that knicked and linear are not the same.

Attachment

- Sma and XhoI have 4.1 KB and 1.2 KB which is expected.
- Sma and NdeI have 1.3 KB and 4.1 KB which is expected

Conclusions:

- All 3 enzymes cut to completion (linearized)
- The cuts are consistent with being pET 28.

Each entry is dated

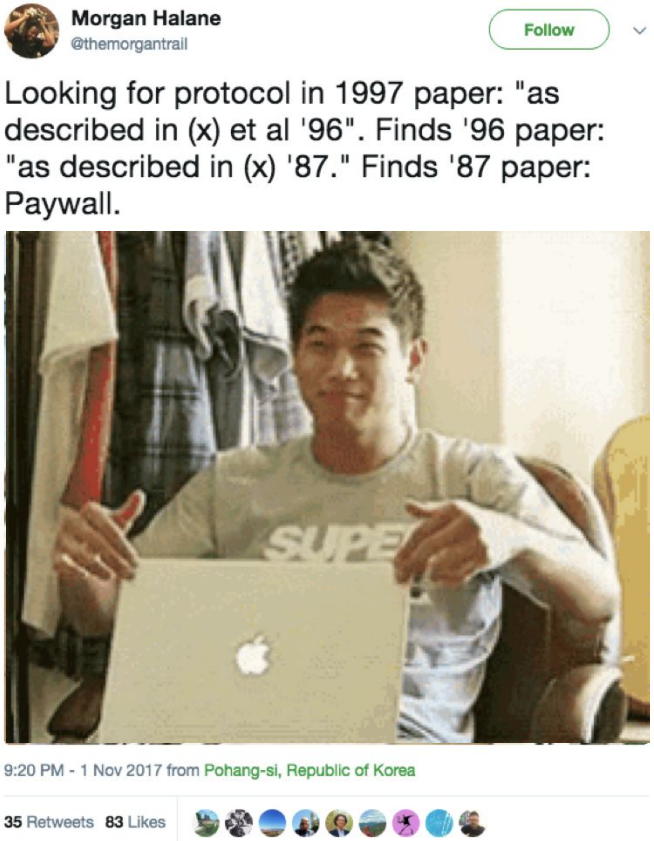
K A Jun 06, 2018 @08:59 AM CDT

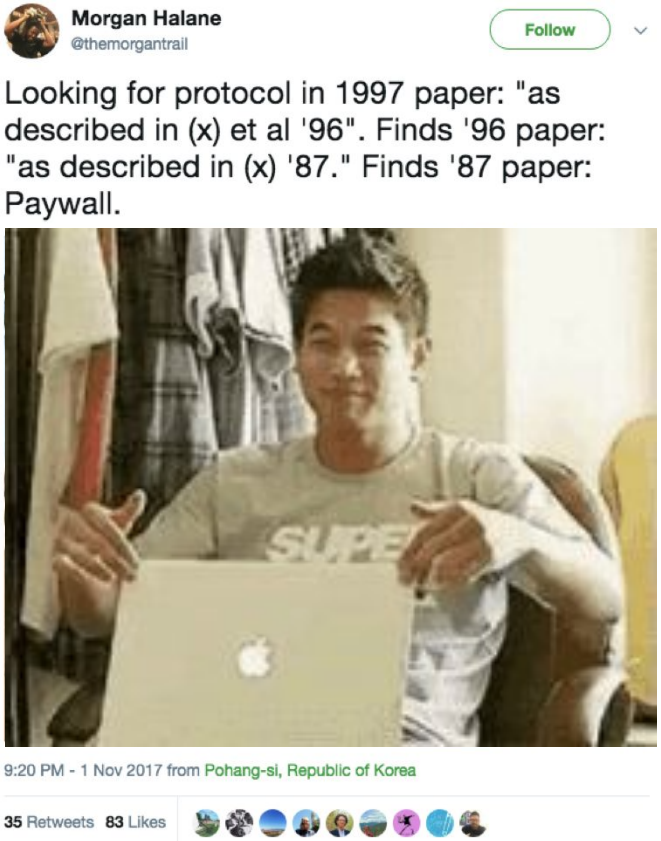
General tips on electronic record keeping

- Back-up data regularly
- Maintain a physical notebook in parallel
- Mobile apps provide added portability
- If using free ELNs, check privacy policies

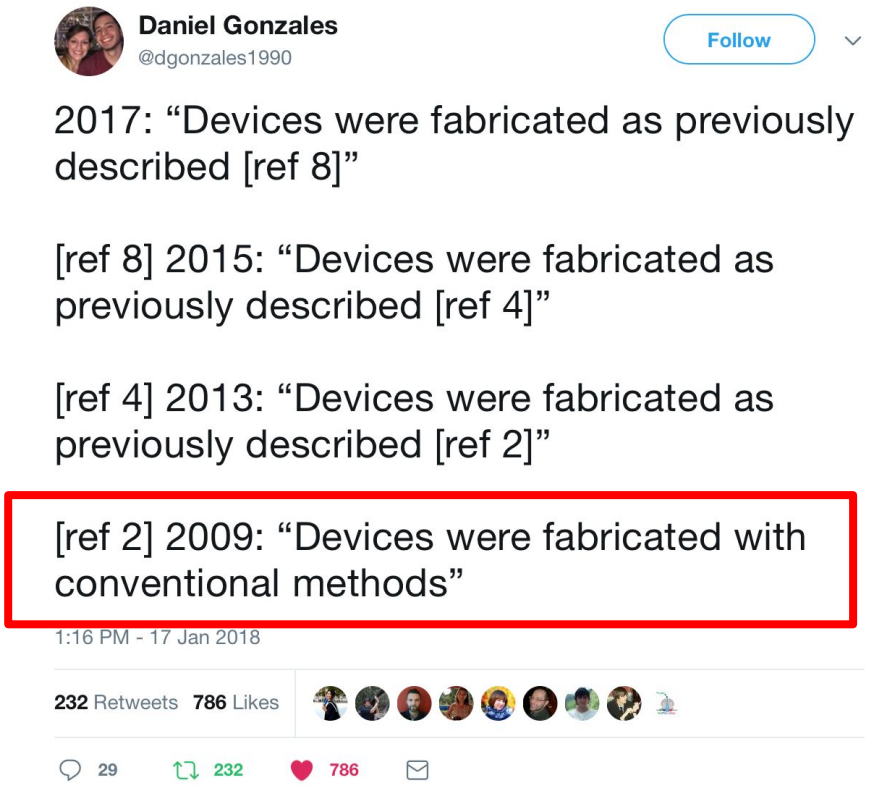
‘Wet lab’ protocol sharing

Description Unavailable




Morgan Halane
@themorgantrail
Follow
Looking for protocol in 1997 paper: "as described in (x) et al '96". Finds '96 paper: "as described in (x) '87." Finds '87 paper: Paywall.
9:20 PM - 1 Nov 2017 from Pohang-si, Republic of Korea
35 Retweets 83 Likes

Description Ambiguous



Description Insufficient

Timothée Poisot Follow
Ecologist. Not that kind of doctor.
Sep 8, 2015 · 2 min read

Step 2—do the rest of the fucking analysis

How to draw an owl

1.

2.

1. Draw some circles

2. Draw the rest of the fucking owl

So when starting a new research project, one can feel like one is trying to draw an owl using the above tutorial. This is because we tend to learn about

How to overcome this problem? - Don't contribute to it!

Methods section could read

We draw the owl on 60.2 gsm white paper of the A4 dimension (210mm by 297mm), using 3H and 6B graphite pencils (Derwent, Cumbria, UK). We did so by looking at owls, and drawing what we saw on paper. This protocol yielded one drawn owl.

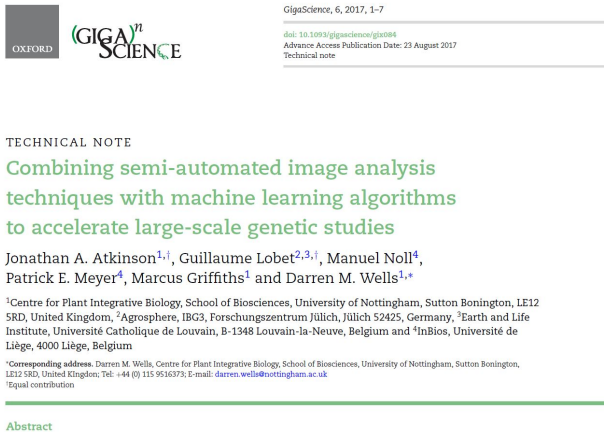
https://medium.com/@tpoi/do-the-rest-of-the-fucking-analysis-8fcef22fd991

Write Detailed Protocols

- Think of a protocol as a brief, modular and self-contained scientific publication.
- Include a 3-4 sentence abstract that puts the methodology in context.
- Include as much detail as possible (Duration/time per step, Reagent Amount, vendor name, Catalog number, Expected result, Safety information, Software package)
- Chronology of steps.
- Notes, recipes, tips, and tricks

https://www.protocols.io/view/how-to-make-your-protocol-more-reproducible-discov-g7vbzn6
https://www.aje.com/en/arc/how-to-write-an-easily-reproducible-protocol/

Share protocols on the right platforms



OXFORD (GIGA)n SCIENCE

GigaScience, 6, 2017, 1–7

doi: 10.1093/gigascience/gix084
Advance Access Publication Date: 23 August 2017
Technical note

TECHNICAL NOTE

Combining semi-automated image analysis techniques with machine learning algorithms to accelerate large-scale genetic studies

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†Equal contribution

Abstract

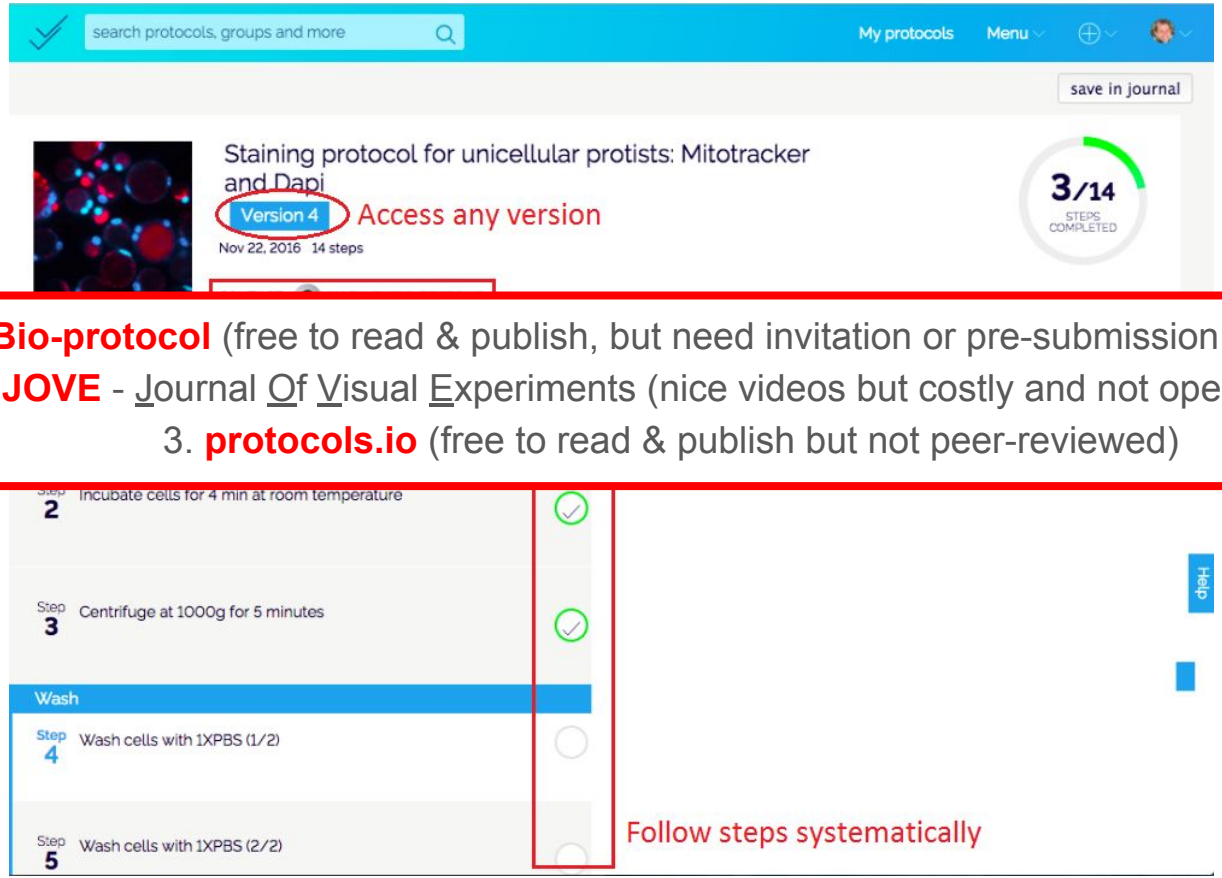
Methods

A detailed version of the protocol described here is available at protocols.io [21].

Availability of supporting source code and requirements

—Project name: PRIMAL, Pipeline of Root Image analysis using MAchine Learning
—Project home page: https://plantmodelling.github.io/primal/
—Operating system(s): platform independent
—Programming language: R
—Other requirements: none
—License: GPL

Share protocols on the right platforms



1. **Bio-protocol** (free to read & publish, but need invitation or pre-submission inquiry)
2. **JOVE** - Journal Of Visual Experiments (nice videos but costly and not open access)
3. **protocols.io** (free to read & publish but not peer-reviewed)

What should Ben do?

Ben is really excited to join a new team that is performing a chemical screen of plant growth regulators on root architecture. However,

- The previous Postdoc started a new job and refuses to respond to his emails.
- The technician on the project was only involved in the data acquisition steps.
- Unfortunately, the lab notebook went missing in a recent move to a new floor.
    - The methods section in a previous paper reads like this -

Materials and Methods
Plants were grown on appropriate media and roots photographed. Images were analyzed using WinRhizo (Arsenault, J-L., et al. 1995) and data presented as graphs.

Identify the problem(s)?

Suggest a solution.

‘Wet lab’ reagent sharing

Problems with wet-lab reagent availability

Scientist creates & publishes on a reagent

Scientist leaves the lab and stores reagent in freezer

???

???

Other scientists request the reagents, but no one remaining remembers where they are

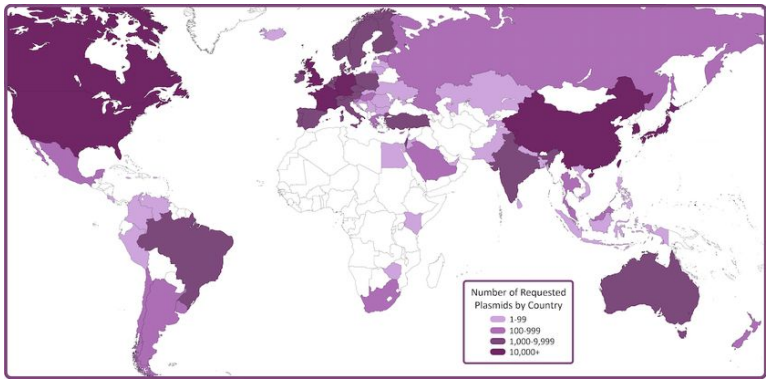
Problems with wet-lab reagent availability

- Wasted time, money, and resources when reagents are recreated
- Mistakes in recreation can lead to spurious results
- Individual labs don't usually have the resources to:
  - Keep track of all reagents created in lab
  - Consistently validate all reagents in the lab
  - Properly label and store all reagents
  - (Legally) distribute all reagents to interested researchers
- Reagents repositories are part of the solution!

Functions of reagent repositories

They:

- Verify reagents
- Curate reagents
- Facilitate and track shipping
- Protect IP



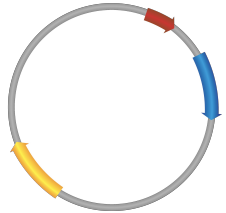
Process is easier if you:

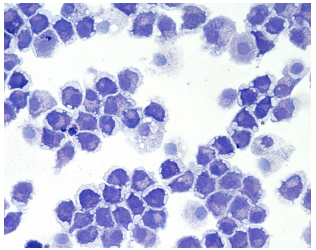
- Record how a reagent was created
- Provided associated publications
- Provided associated protocols

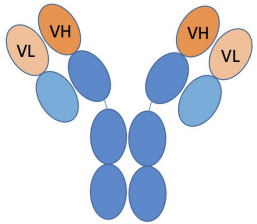
(All of these are facilitated by other tools discussed in this workshop)

Examples of Reagent repositories

- Addgene
- DNASU
- ATCC
- NCI Mouse Repository
- Coriell Institute
- ABRC
- The Bloomington Drosophila Stock Center
- Developmental Studies Hybridoma bank







Incentivizing reagent sharing

Direct

- Archiving
- Reducing time spend sending out reagents
- Occasional monetary benefits

Indirect

- Creation of educational content
- Direct promotion
- Analysis of reagent distribution

Addgene: The nonprofit plasmid repository

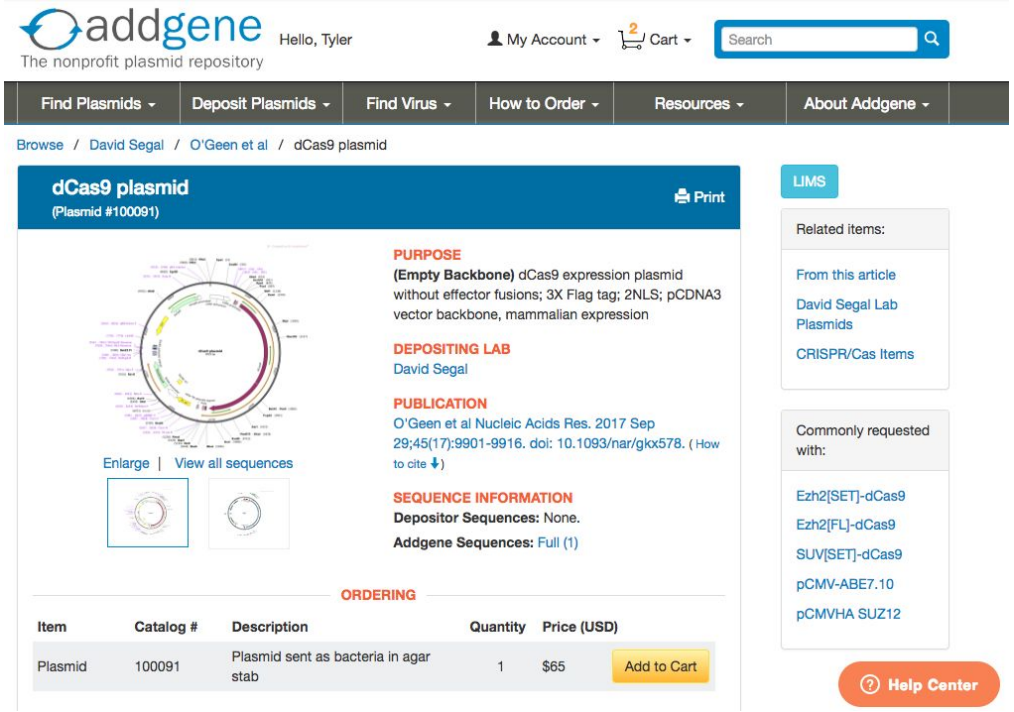
Goal: To accelerate science by improving access to research materials and information

Issues Addressed: Difficulties in obtaining, verifying, and using plasmids from other labs

Audience: Academic and nonprofit institutions doing biology research and using plasmids

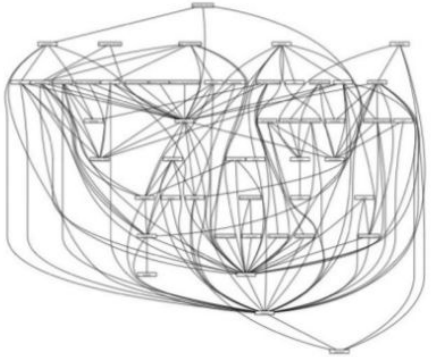
Services:

- Stores and distributes plasmids and viral vectors
- Verifies plasmids and viral vectors through DNA sequencing with some functional testing
- Collates/curates information about plasmids and viral vectors
- Produces and freely distributes educational content to make it easier for scientists to learn about and use new technologies



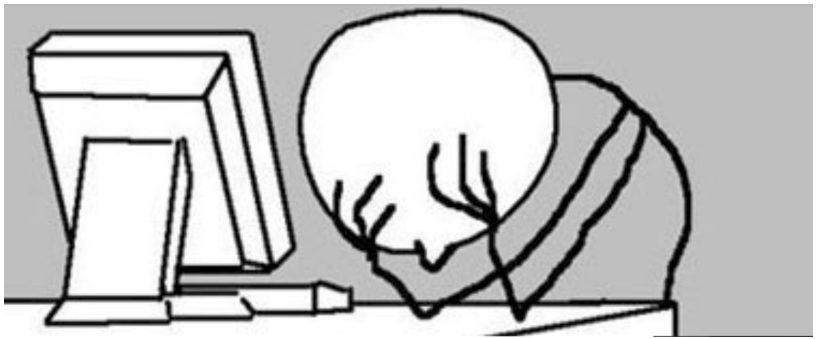
Bioinformatic tools

Dependency hell



What version of the program, data etc… did I use?

Why did I do this?



https://software-carpentry.org/

‘Bioinformatic data skills’ Vincent Buffalo

Notebooks

- Keep track of analysis
- Interactive coding
- Interactive data exploration
- Imbedded visualization
- Easy access to docstrings
- Mix of code and documentation

https://jupyter.org/documentation

https://www.rstudio.com/

Notebooks

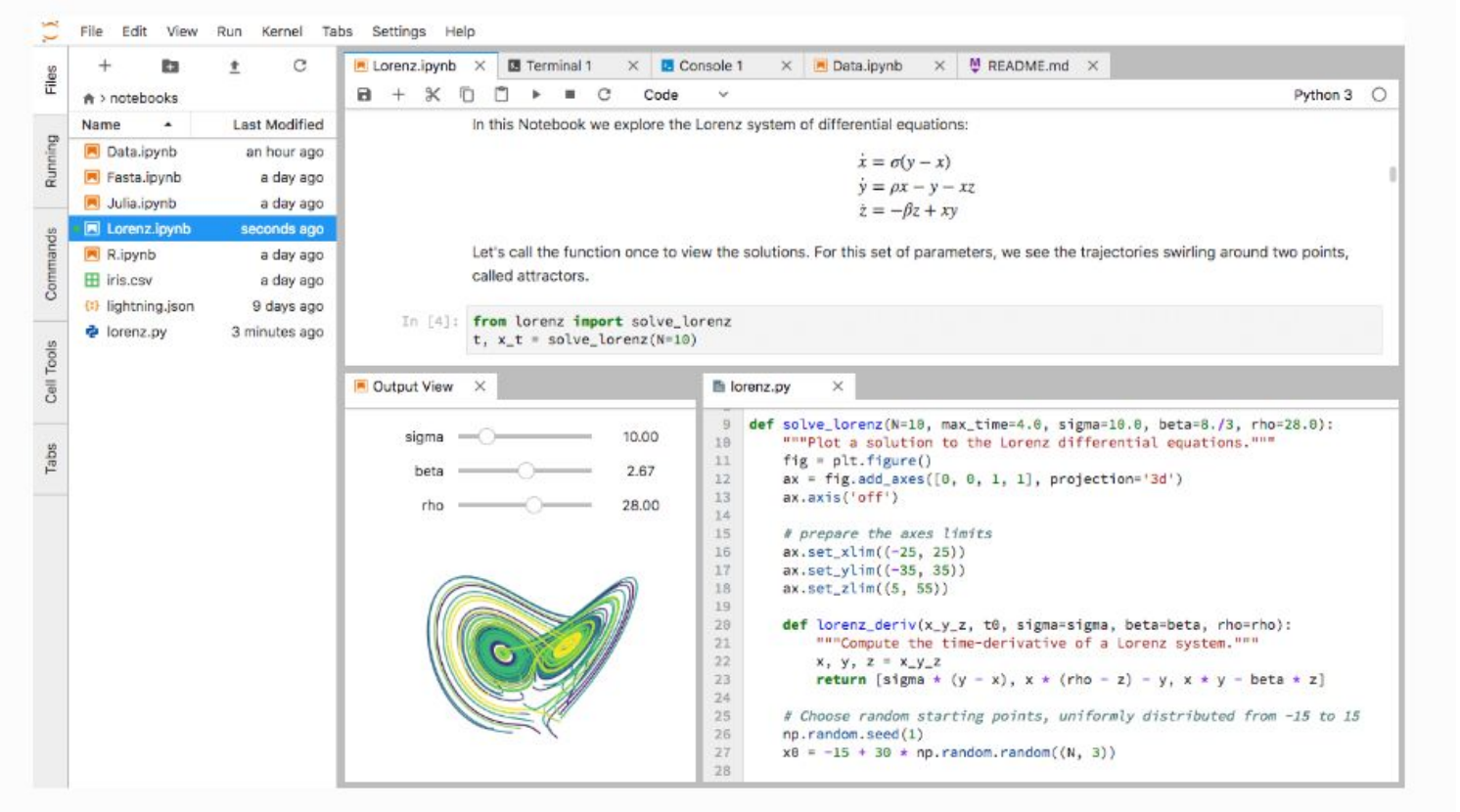
- Keep track of analysis
- Interactive coding
- Interactive data exploration
- Imbedded visualization
- Easy access to docstrings
- Mix of code and documentation

RStudio®

- Over 40 programming languages
- Easily shared
- Widgets
- Interactive plots
- Run remotely on server

https://jupyter.org/documentation

https://www.rstudio.com/



https://jupyter.org/documentation

https://www.rstudio.com/

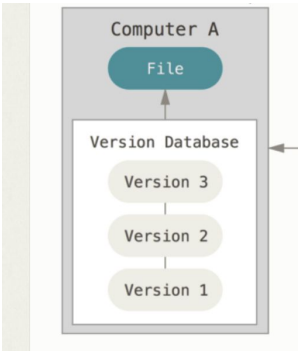
Version control

- Records changes
- Keeps track of change history
- Illustrates changes between versions
- Lets you share your code easily
- Lets you collaborate on your code more easily

https://git-scm.com/doc

http://smutch.github.io/VersionControlTutorial//

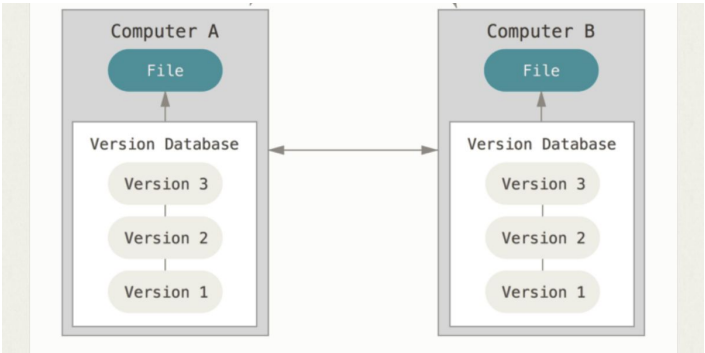
Version control



https://git-scm.com/doc

http://smutch.github.io/VersionControlTutorial//

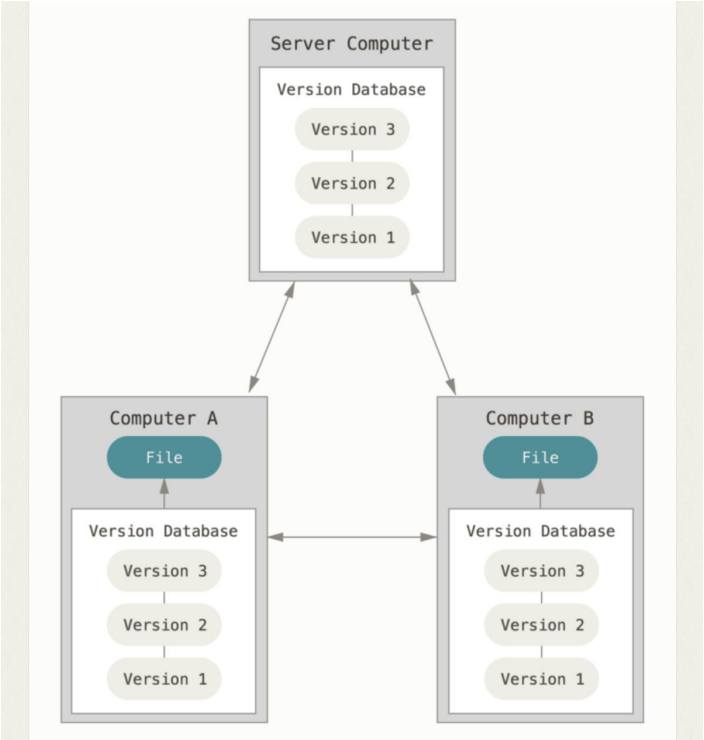
Version control



https://git-scm.com/doc

http://smutch.github.io/VersionControlTutorial//

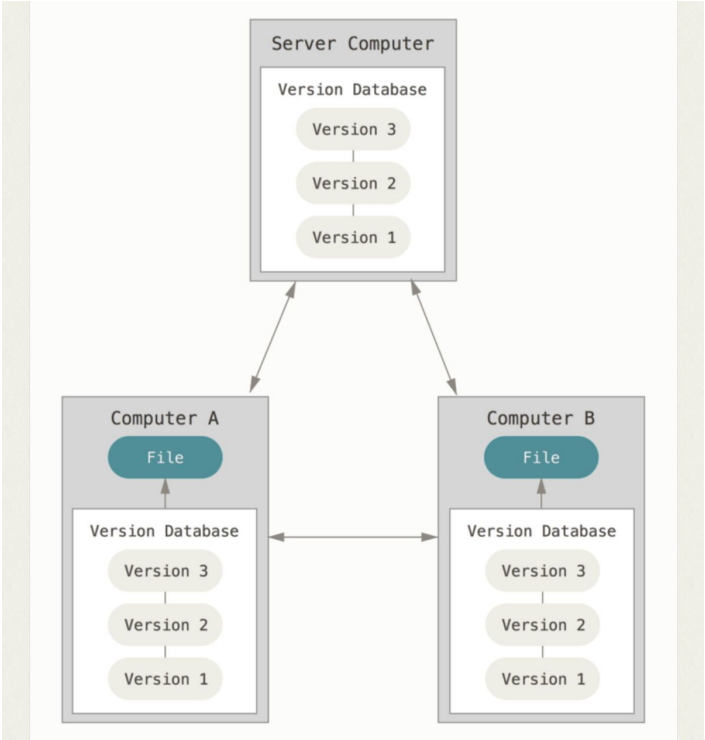
Version control



https://git-scm.com/doc

http://smutch.github.io/VersionControlTutorial//

Version control



GitHub

git

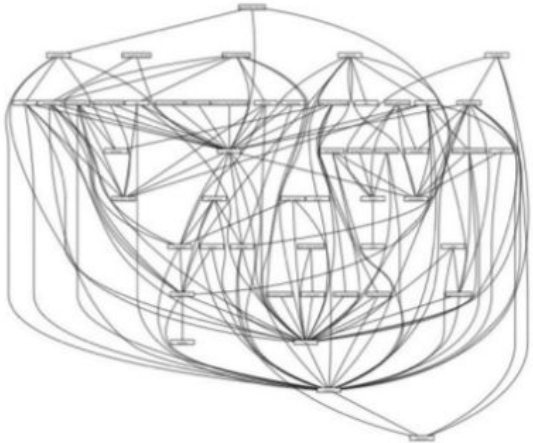
Google docs does history tracking.

https://git-scm.com/doc

http://smutch.github.io/VersionControlTutorial//

How to I install all these different software packages???

Dependency hell



Version conflict

Package, dependency, and environment manager

- Handles installs and dependencies
- Allows for multiple independent environments
- Easily configurable
- Allows for manual installs as well
- Runs on all three major systems
- Open source

- You can package your own work and contribute

https://conda.io/docs/

https://bioconda.github.io/

It is really that simple….

```
conda install bwa
```

Or a new environment can be created:

```
conda create -n aligners bwa bowtie hisat star
```

…. most of the time

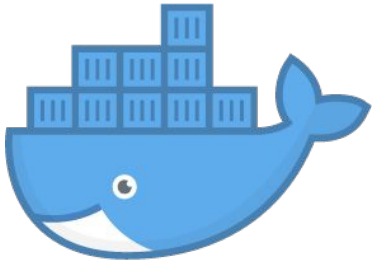
BIOCONDA®

https://conda.io/docs/

https://bioconda.github.io/

Containers

Biocontainers



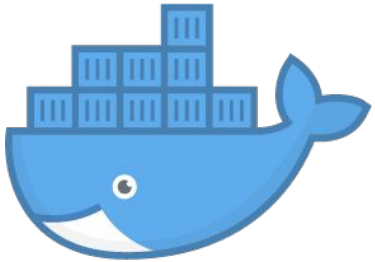
Docker runs images as containers that are

- self contained with all code, programs, libraries included. No subsequent installation required.
- Isolated
- Portable including dissemination
- Lightweight

https://docs.docker.com/

Containers

Biocontainers



Docker

Get local blast

```
$ docker pull biocontainers/blast
```

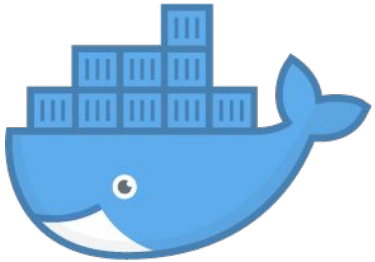
Run local blast

```
$ docker run biocontainers/blast blastp -help
```


https://docs.docker.com/

http://biocontainers.pro

Containers



binder

Turns a GitHub repo with data and notebooks into a collection of interactive notebooks run in the cloud executable

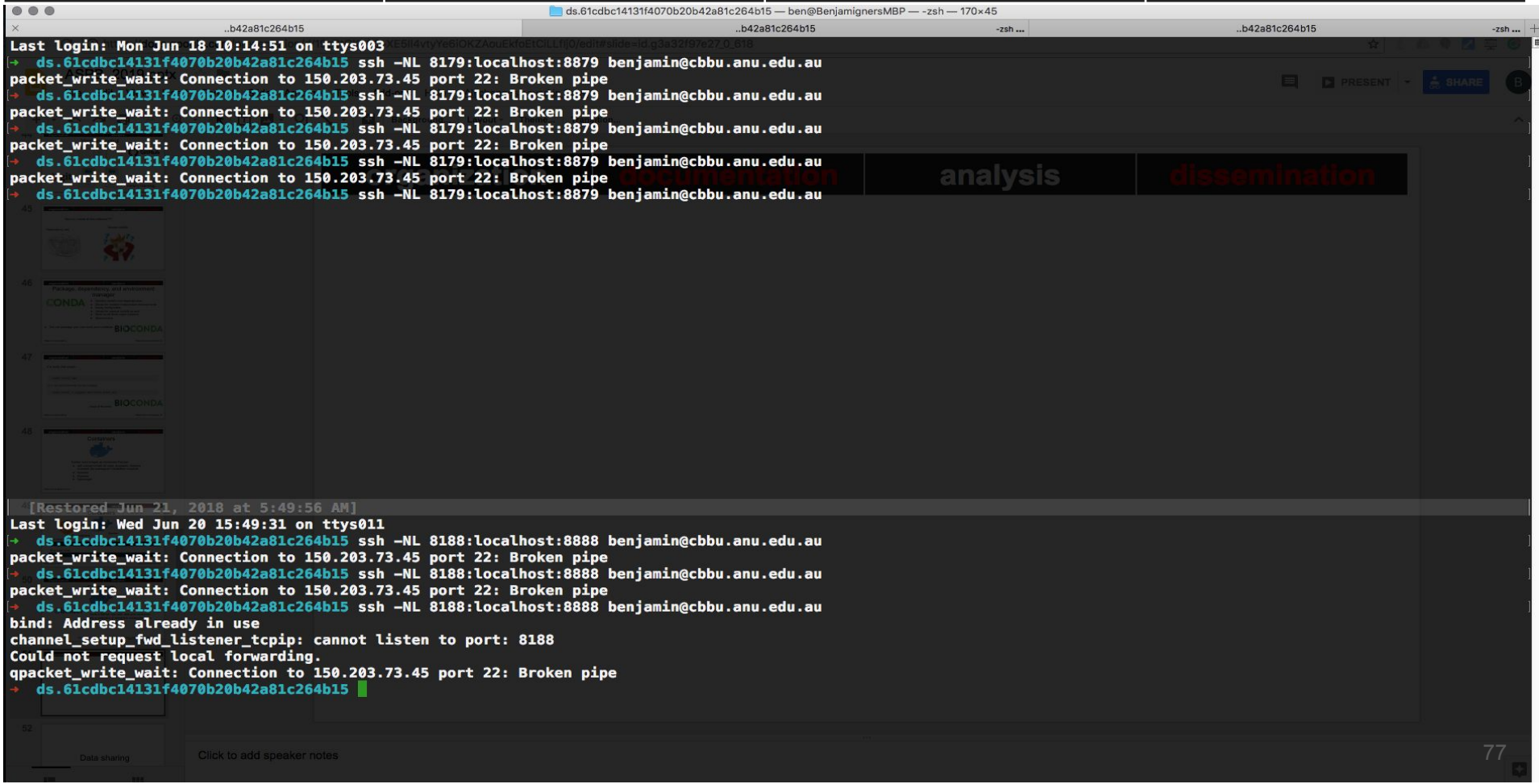
https://mybinder.org/

CODE OCEAN BETA

Configuration, preservation, & reuse of executable code using containers for researchers

https://tinyurl.com/jupyter-binder2-0

https://tinyurl.com/eLife-binder2-0


ds.61cdbc14131f4070b20b42a81c264b15 — ben@BenjamignersMBP — -zsh — 170×45
..b42a81c264b15
..b42a81c264b15
-zsh ...
..b42a81c264b15
-zsh ...
Last login: Mon Jun 18 10:14:51 on ttys003
ds.61cdbc14131f4070b20b42a81c264b15 ssh -NL 8179:localhost:8879 benjamin@cbbu.anu.edu.au
packet_write_wait: Connection to 150.203.73.45 port 22: Broken pipe
ds.61cdbc14131f4070b20b42a81c264b15 ssh -NL 8179:localhost:8879 benjamin@cbbu.anu.edu.au
packet_write_wait: Connection to 150.203.73.45 port 22: Broken pipe
ds.61cdbc14131f4070b20b42a81c264b15 ssh -NL 8179:localhost:8879 benjamin@cbbu.anu.edu.au
packet_write_wait: Connection to 150.203.73.45 port 22: Broken pipe
ds.61cdbc14131f4070b20b42a81c264b15 ssh -NL 8179:localhost:8879 benjamin@cbbu.anu.edu.au
[Restored Jun 21, 2018 at 5:49:56 AM]
Last login: Wed Jun 20 15:49:31 on ttys011
ds.61cdbc14131f4070b20b42a81c264b15 ssh -NL 8188:localhost:8888 benjamin@cbbu.anu.edu.au
packet_write_wait: Connection to 150.203.73.45 port 22: Broken pipe
ds.61cdbc14131f4070b20b42a81c264b15 ssh -NL 8188:localhost:8888 benjamin@cbbu.anu.edu.au
packet_write_wait: Connection to 150.203.73.45 port 22: Broken pipe
ds.61cdbc14131f4070b20b42a81c264b15 ssh -NL 8188:localhost:8888 benjamin@cbbu.anu.edu.au
bind: Address already in use
channel_setup_fwd_listener_tcpip: cannot listen to port: 8188
Could not request local forwarding.
qpacket_write_wait: Connection to 150.203.73.45 port 22: Broken pipe
ds.61cdbc14131f4070b20b42a81c264b15
Click to add speaker notes
77

Data sharing

Data sharing

What to share?

- Share research data and code that is necessary to **validate findings** & **reproduce results** of research outputs
- Share data and code that might be **valuable** to other researchers or policy-makers
- Share data and code which **cannot be (easily) re-generated**

Why share?

- Funder or publisher mandates
- Citation benefits (Piwowar 2013, https://doi.org/10.7717/peerj.175)
- Preserve long-term access to data

How to share?

- Choose open, persistent, and non-proprietary file formats
- Create and share documentation to enable reuse
- Include data citations of source data
- Create rich metadata

Data sharing

- Use a data repository, not your website!
  - Repositories provide
    - Persistent identifiers for your data like a DOI
      - Unique and citable
      - Prevents “link rot”
    - Persistent access
    - Preservation
    - Backup
    - Management of access
    - Versioning
    - Licensing

Specify a data licence:

- Consider Creative Commons licenses for data and text, either CC-0 or CC-BY. Guidance on data licenses from the Digital Curation Center: http://www.dcc.ac.uk/resources/how-guides/license-research-data

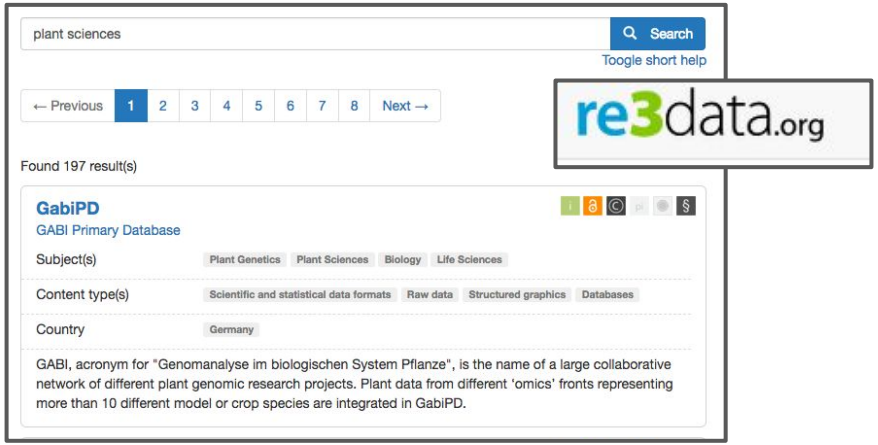
Specify a code licence:

- Consider an open source license such as the MIT, BSD, or Apache license. Guidance on software licenses from Karl Broman: http://kbroman.org/steps2rr/pages/licenses.html and Open Source Initiative: https://opensource.org/licenses

Data sharing

Identify mandated or disciplinary repository:

- Funder specified repository
- Institutionally specified data repository
- Domain or discipline-specific data repository
  - Find and compare disciplinary repositories using the Repository of Research Data Repositories https://www.re3data.org/



In addition to a specified data repository, you can make a deposit to a general purpose repository:

- DataDryad http://datadryad.org/ (curated digital repository; free to access, $120 to publish dataset up to 20GB)
- Figshare https://figshare.com/ (free digital repository, 5GB per file limit)
- Zenodo https://zenodo.org/ (free digital repository; 50GB per dataset limit)

DRYAD

figshare
credit for all your research

zenodo

Image handling and analysis

Challenges:

- reproducibility of image capture
- manage data sets
- accurately represent 3D data in a 2D format
- reproducibility of image analysis

Reproducibility of image capture

Issues

- Variability in fluorescent lines/staining
- Variability in laser power detector sensitivity
- Differing sensitivity of markers
- Variations in expression due to time of day, developmental stage, growth conditions

Possible solution
More detailed methods?

Manage image data

Issues

- Storing images and meta data with sufficient information that on how they were captured, what they are and how they were processed.
- Files are often very large and in a commercial format.

Possible solutions

https://www.openmicroscopy.org/

https://www.openmicroscopy.org/omero/

Accurately represent 3D data in a 2D format

Issues

- showing single slices is not representative
- max projections vs average projections give different impression

Possible solutions

- Share raw data
- movies of time-course data
- movies to show full z-stacks

Reproducibility of image analysis

Issues

- can another researcher reproduce your analysis?

Possible solution

https://fiji.sc/

Fiji/ ImageJ - open source software for image analysis

- Enables automated renaming conversion of image formats to reduce human error
- Quantitative image analysis
- Use huge number of plugins/macros available or write own
- Enables sharing of analysis pipeline with the data
- Interacts with OME

MorphoGraphX is an open source application for the visualization and analysis of 4D biological datasets. Developed by researchers, it is primarily used for the analysis and quantification of 4D live-imaged confocal data

- Enables projections of stacks, movies and 3D rendering of confocal stack
- Quantification of geometry and signal intensity
- Records processing that occurs with the data
- Can automate segmentation with macros
- Can share pipeline of image analysis to enable reproducibility of the analysis

http://www.mpipz.mpg.de/MorphoGraphX

Data Analysis and Visualization

Data presentation is the foundation of our collective scientific knowledge…

Figures are especially important. They often show data for key findings.

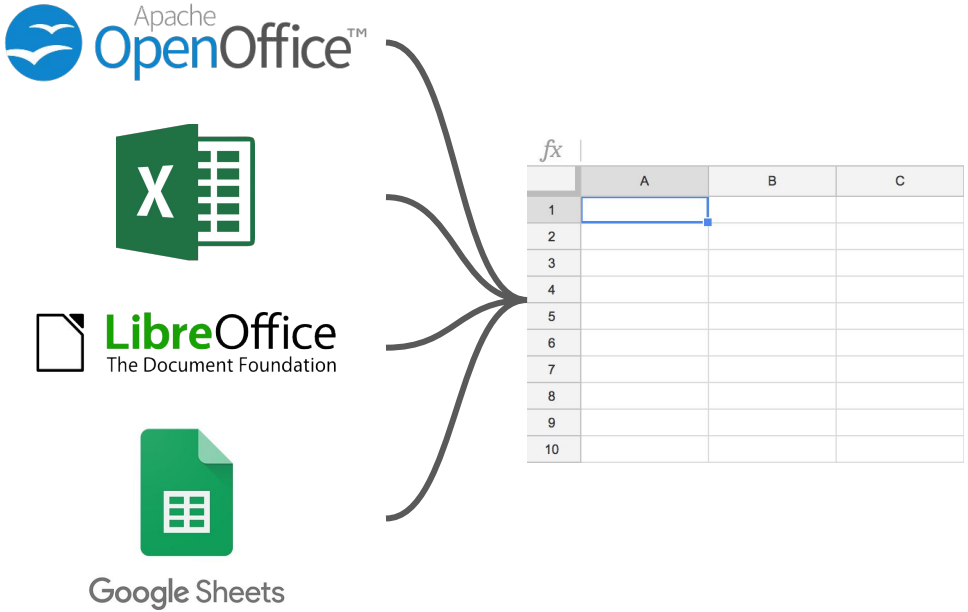
What is good DataViz?

Effective figures should:

1. Immediately convey information about the study design
2. Illustrate important findings
3. **Allow the reader to critically evaluate the data**

Weissgerber et al.
10.1074/jbc.RA117.000147

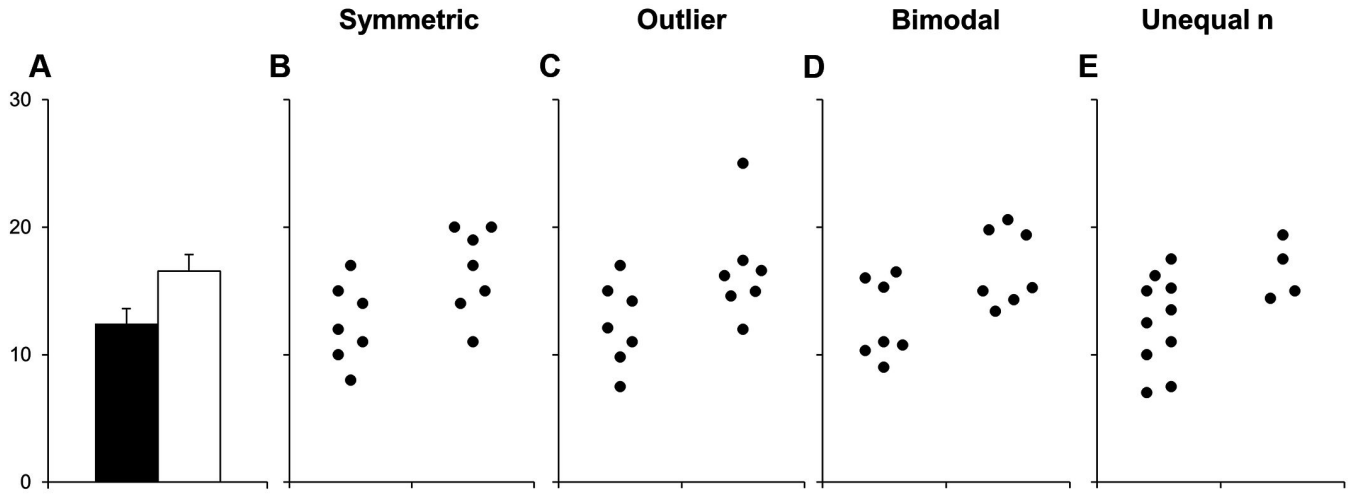
The usual way and its flaws



Issues:

- Reproducible Workflows?
  - Problems can be avoided by using macros or dashboards
  - However, who uses these?
- Excel Renames Genes
  - Ziemann et al., 2016 - https://doi.org/10.1186/s13059-016-1044-7
  - 20% of papers in leading genomic journals contain gene list errors
- Default Plots are often Bar Charts and Line Plots

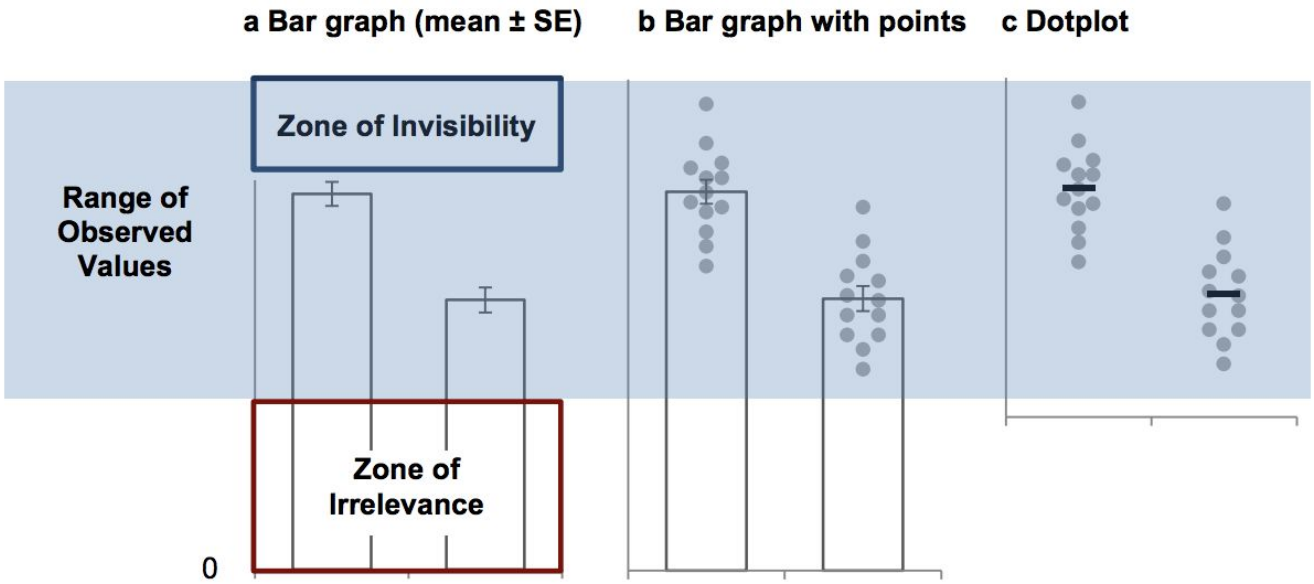
Why does DataViz matter for reproducibility?



Test	p value			
T-test: Equal var.	0.035	0.050	0.026	0.063
T-test: Unequal var.	0.035	0.050	0.026	0.035
Wilcoxon	0.054	0.073	0.128	0.103

Weissgerber et al., 10.1371/journal.pbio.1002128

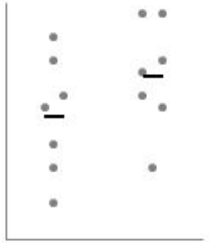
Why does DataViz matter for reproducibility?



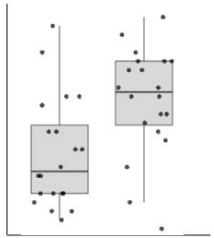
Bar Charts Don't Allow You to Critically Evaluate Continuous Data

Weissgerber et al., 2017 JBC, http://www.jbc.org/content/292/50/20592.full

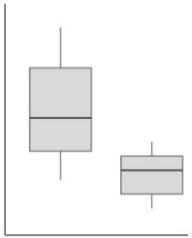
How to Choose the Right Plot



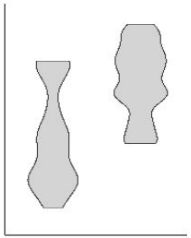
Dotplot



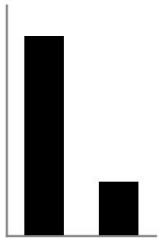
Boxplot with points



Boxplot



Violin plot (with or without points)



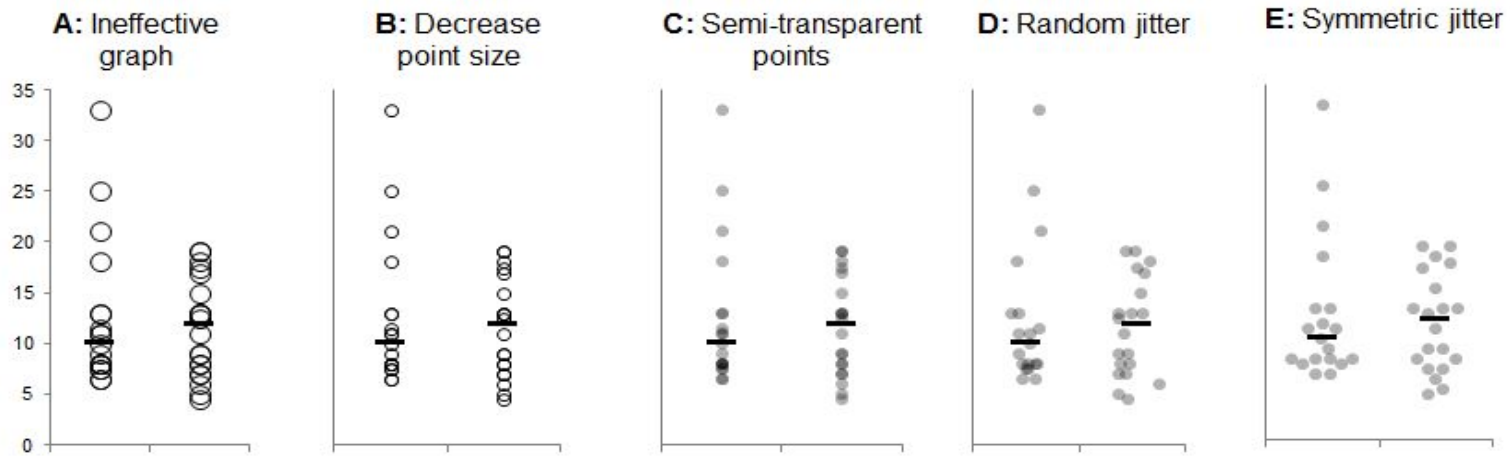
Bar graph

	Dotplot	Boxplot with points	Boxplot	Violin plot (with or without points)	Bar graph
Outcome variable	Continuous	Continuous	Continuous	Continuous	Counts & proportions
Sample size	Small	Medium	Large	Medium to Large	Any
Data distribution	Any	Any	Do not use for bimodal data	Any	N/A

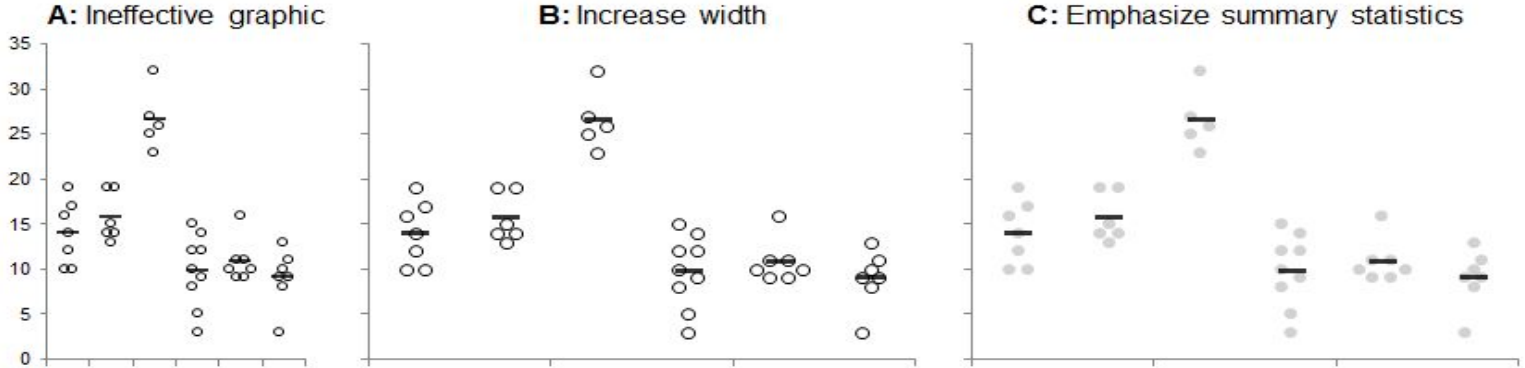
Making Effective Dotplots

Tracey Weissgerber Personal Communication

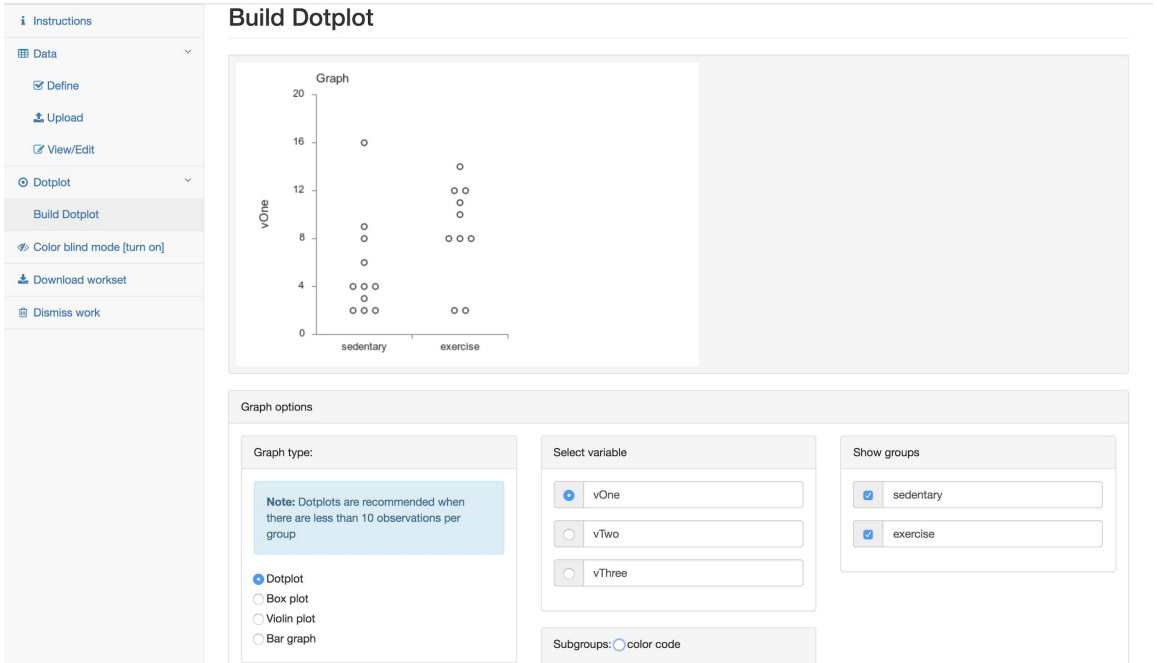
Step 1: Make all data points visible



Step 2: Emphasize summary statistics



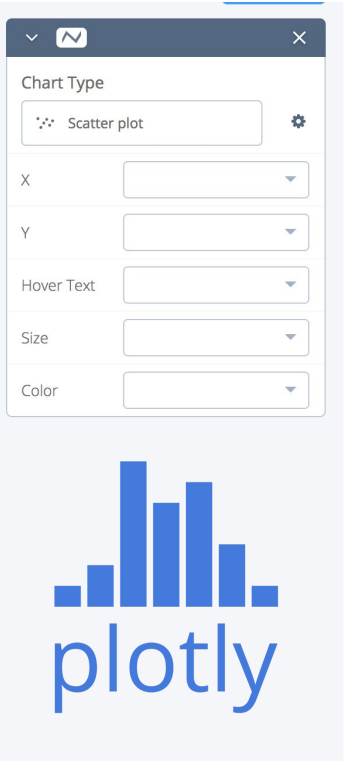
One Step Further

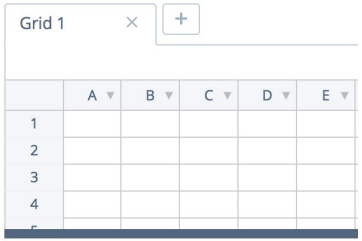


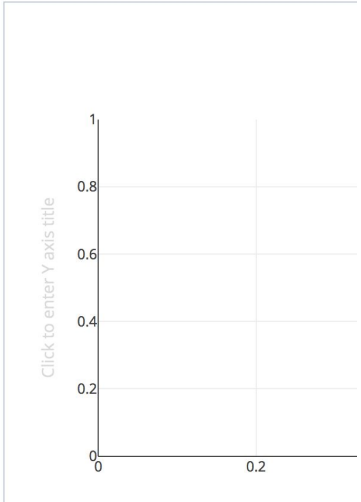
Interactive Dot Plot - http://statistika.mfub.bg.ac.rs/interactive-dotplot/

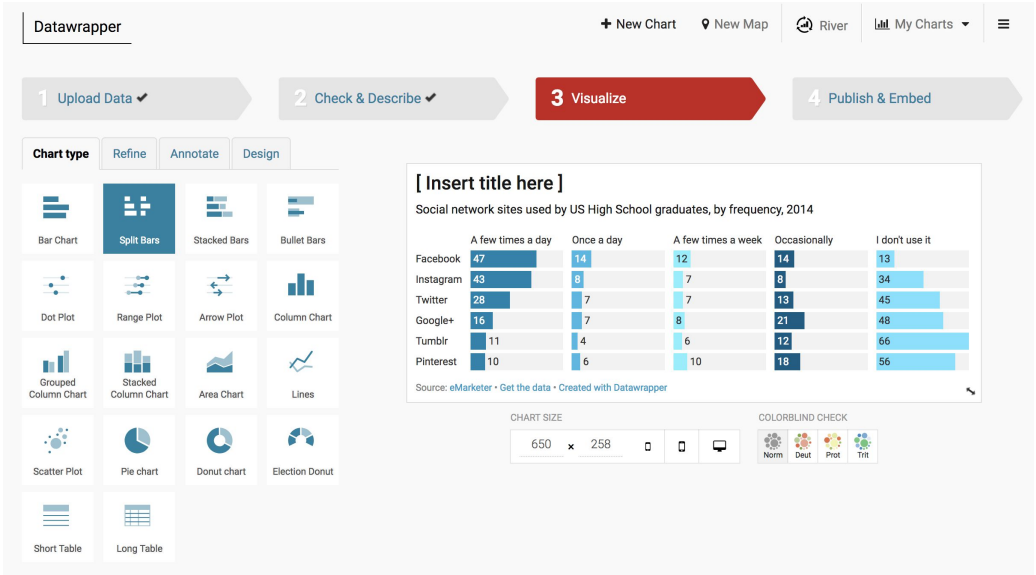
Interactive Line Graph - http://statistika.mfub.bg.ac.rs/interactive-linegraph/

Some Intermediate Options









https://plot.ly/create/#/

https://www.datawrapper.de/

Which programming language should I use?

- Select a language that is used in your lab or community
- *Select a general purpose language such as Python to start with if you don't have a specific problem. That way you learn basic programming skills, which allows you to switch to other languages more easily, and you can tackle different problems. You usually learn multiple languages anyway.

Programming Languages

- Anaconda (Distribution)
- Numpy & Pandas (Data Wrangling)
- Scipy (Higher Math)
- Matplotlib (Basic Graphs)
- Seaborn, Bokeh, Altair, Plotly (Advanced Statistical & Interactive Graphs)
- Jupyter notebook / lab (Interactive Notebook)

- tidyverse (Distribution)
- dplyr & tidyr (Data Wrangling)
- ggplot / ggplot2 (Basic Graphs)
- shiny / RMarkdown (Interactive Notebook)
- RStudio (Interactive Notebook)

Dealing with Data

- **Provide Open-Source Data** (*Rule 2 of Enable Multi-site Collaborations through Data Sharing*)
- **Keep Raw Data Raw** (*Rule 3 of Digital Data Storage*)
- **Store Data in Open Formats** (*Rule 4 of Digital Data Storage*)
- **Data Should Be Structured for Analysis** (*Rule 5 of Digital Data Storage*)
- **Data Should Be Uniquely Identifiable** (*Rule 6 of Digital Data Storage*)
- **Link Relevant Metadata** (*Rule 7 of Digital Data Storage*)
- **Have a Systematic Backup Scheme** (*Rule 9 of Digital Data Storage*)
- **Archive The Data Appropriately**

Summary

Reproducible research practices enable you to:

- Organize experiments productively
- Accurately analyze results
- Share results with future researchers
- Share techniques
- Share reagents with future researchers
- Accelerate science!

The tools discussed here should provide you with the framework to make you research more reproducible and will save you time and resources in the long term

Contributors

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What is one thing that you can do today to start making your research more reproducible?

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