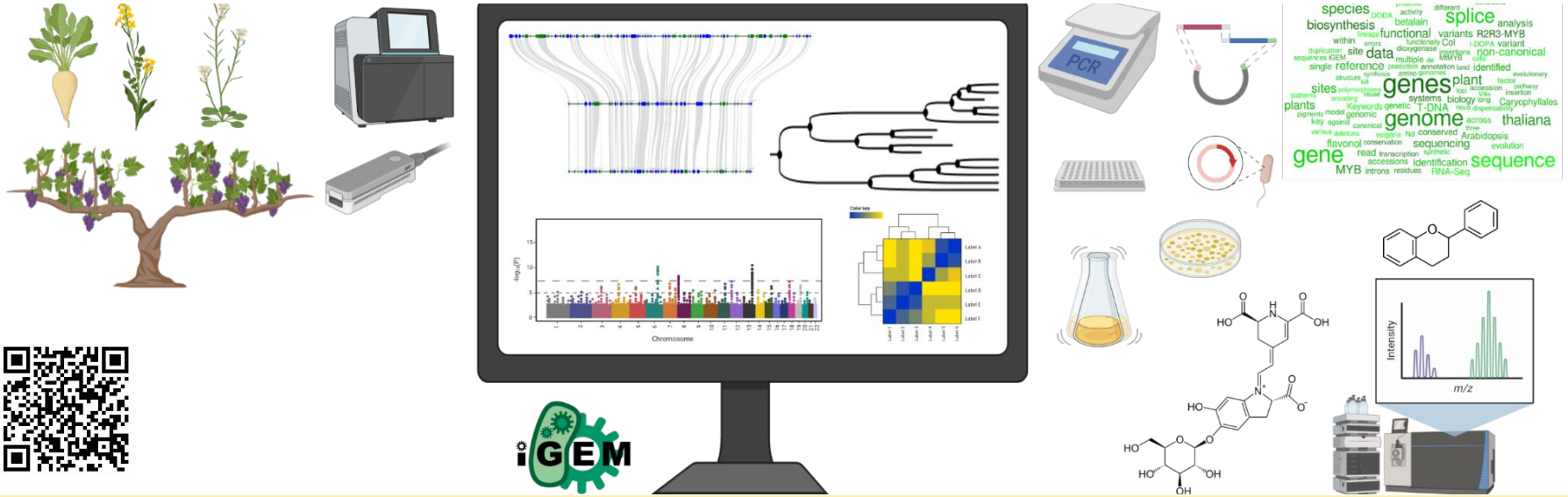
Technische Universität Braunschweig



Introduction to data literacy in genomics

Prof. Dr. Boas Pucker and Katharina Wolff
(Plant Biotechnology and Bioinformatics)

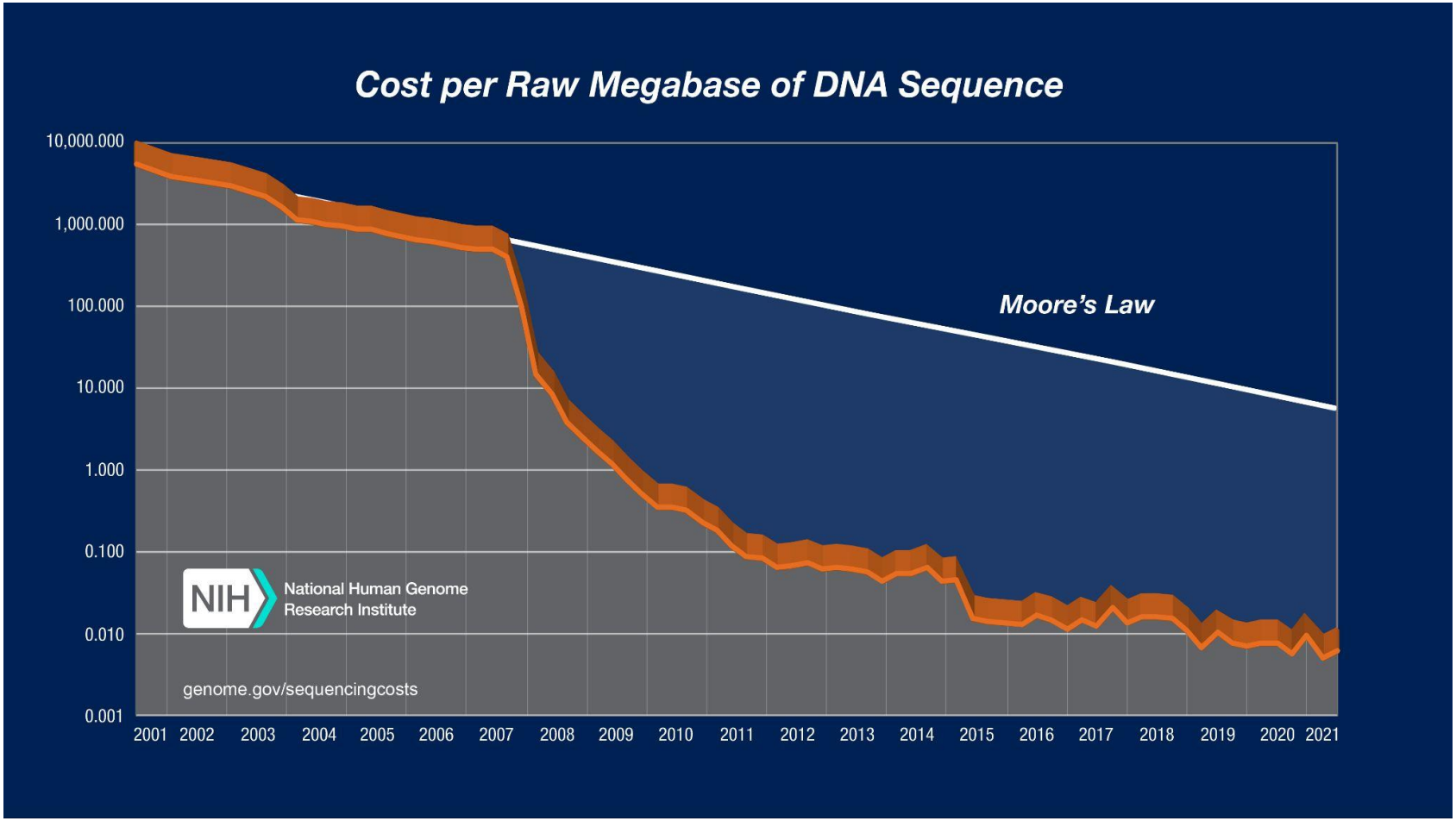
Availability of slides

- All materials are freely available (CC BY) - after the lectures:
  - StudIP: Data Literacy in Genomics
  - GitHub: https://github.com/bpucker/teaching

- Questions: Feel free to ask at any time

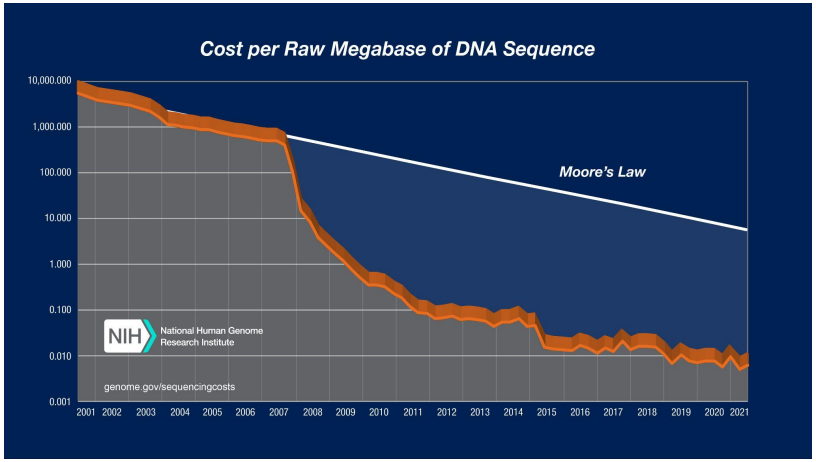
- Feedback, comments, or questions: b.pucker[a]tu-bs.de

‘Big Data’


Cost per Raw Megabase of DNA Sequence
10,000.000
1,000.000
100.000
10.000
1.000
0.100
0.010
0.001
Moore’s Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021

Reasons for growth of databases

- Data generation costs are dropping
  - Rapid sequencing technology development
  - Resolution of pictures is increasing
  - Robotics supports data acquisition
- Data storage capacities are increasing
- Potential of data reuse is recognized



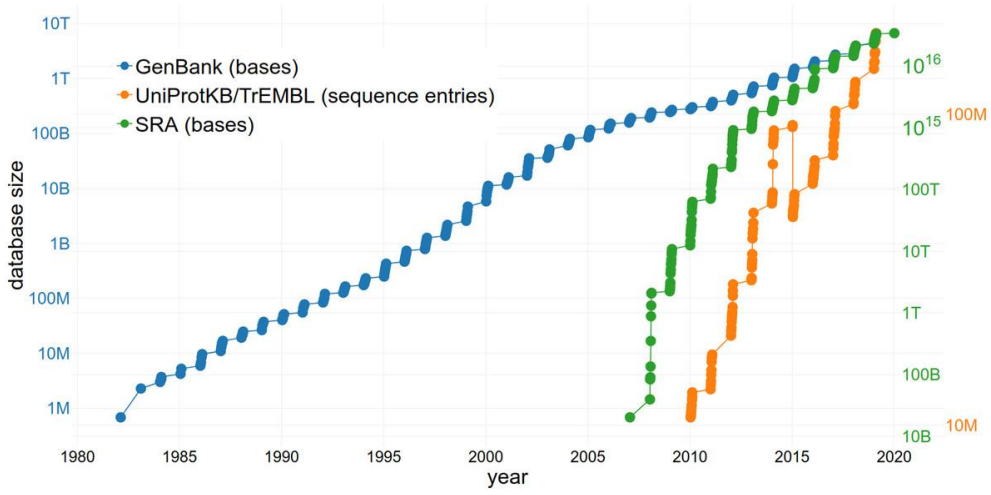
What is Data Literacy?

Data Literacy

- ‘Competency to handle data properly’
- ‘Read, understand, create, communicate data as information’ (wikipedia)

Why is Data Literacy important?

- We live in a world of data: important for science, business, and society
- Objective: data informed decision making
- Sizes and complexity of data sets are increasing (‘BigData’)
- Data is accumulating over time



Sielemann et al., 2020: 10.7717/peerj.9954

Why is Data Literacy important for you?

- Data (interpretation) is valuable
- Data scientists are needed
- Combination of different fields is particularly powerful

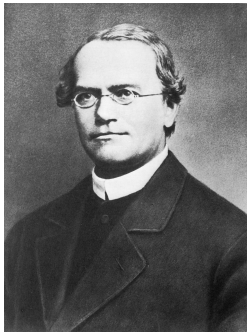
https://doi.org/10.1515/jib-2019-0005

More definitions

- Data: collection of observations
  - 2020: 312 red flowers; 95 white flowers
  - 2021: 298 red flowers; 98 white flowers

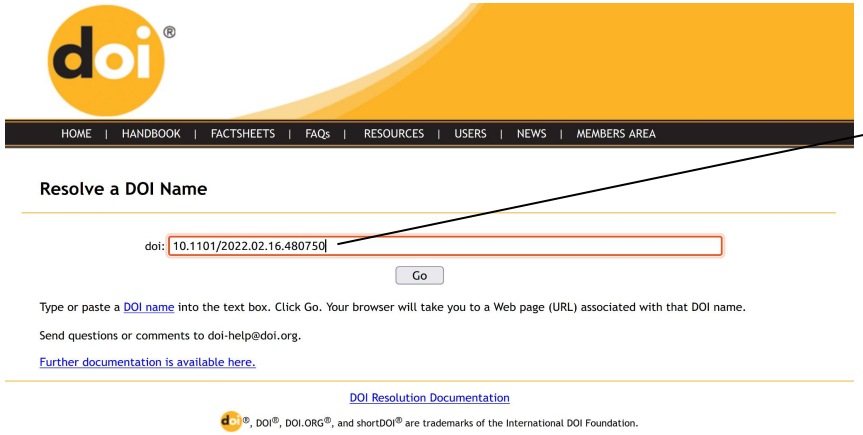
- Knowledge: accumulation of facts and data
  - Ratio of red flowers to white flowers is 3:1

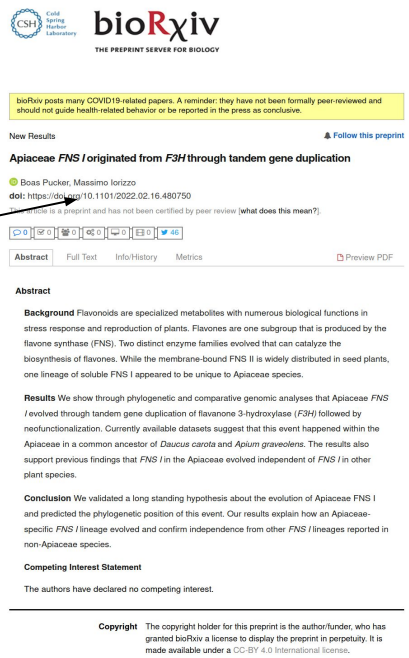
- Insights: grasping the underlying nature of knowledge; understanding general concepts
  - Flower color has a genetic basis
  - Red flower allele dominant over white flower allele



Digital Object Identifier (DOI)

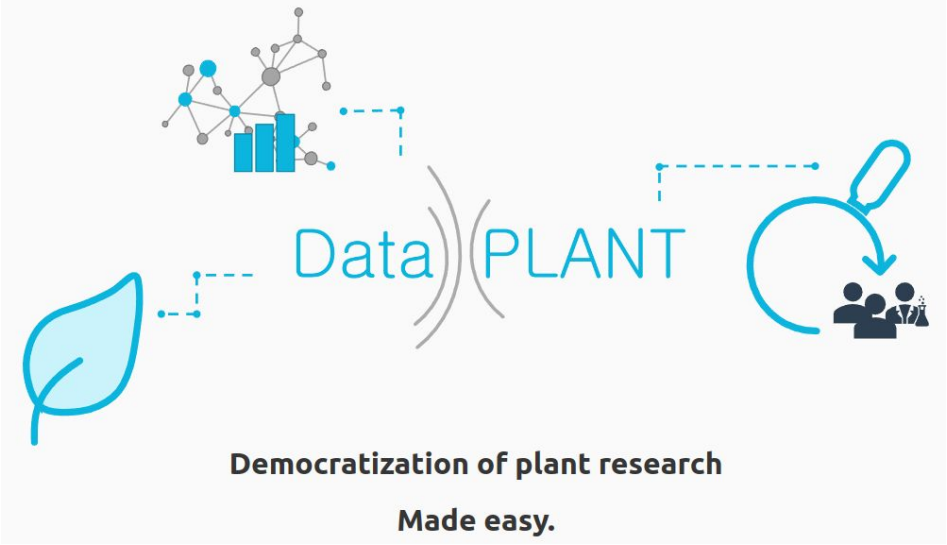
- DOI = Digital Object Identifier
- Unique and short way to point to a publication or a data set
- How to resolve a DOI? https://dx.doi.org/





Data management

- Larger data sets require more efficient data management
- Data management plans required in project proposals
- Many services and organizations emerging (NFDI)



https://nfdi4plants.de/

Data protection and data security

- Data protection is becoming a huge issue in the EU
- Avoid any personal data in your research data sets
- Data security is gaining relevance
- German universities are frequently attacked:
  - Gießen (2019): offline for weeks + 1.7 million direct costs
  - HHU Düsseldorf (2020): ransom attack on clinic
  - Bochum, Dresden, Freiburg, Berlin

https://ec.europa.eu/info/law/law-topic/data-protection/data-protection-eu_en

Electronic Laboratory Notebooks (ELN)

- Links between data sets and documentation
- Avoids repetitive documentation (copy&paste); version control
- Automatic search
- No issues with handwriting
- Quick and regular backups possible
- Accessible from everywhere
- Collaborative

Technical solutions for electronic lab notebooks

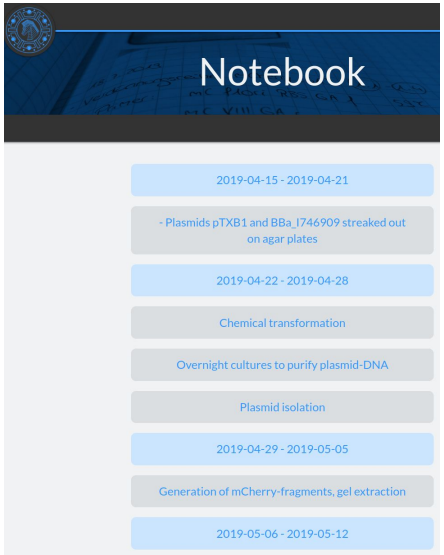
- Simple wiki page

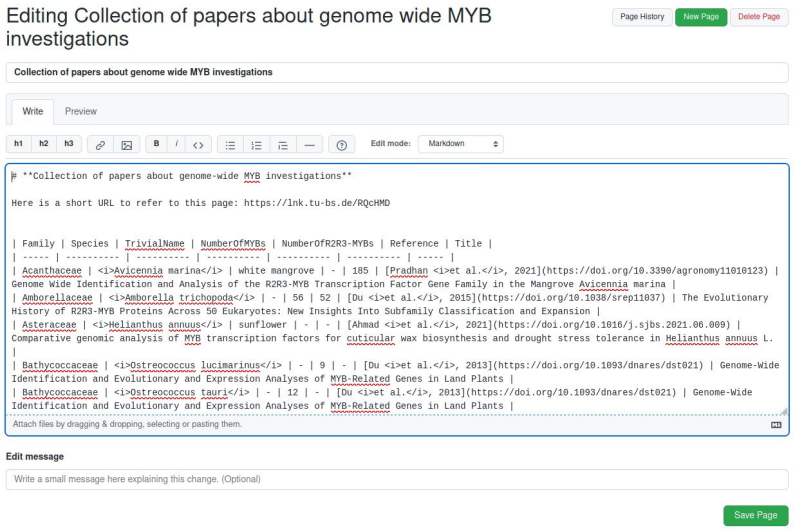
- Dedicated systems developed by research institutions
  - GABI-Kat LIMS
  - Chemotion
  - e!DAL
  - gitlab

- Commercial offers
  - Benchling
  - Dotmatic's
  - Signals Notebook (Perkin Elmer)
  - LabArchives

Example: wiki

- Electronic lab notebook for collaborative learning
- iGEM wiki to document team projects
- Github wiki to document tools

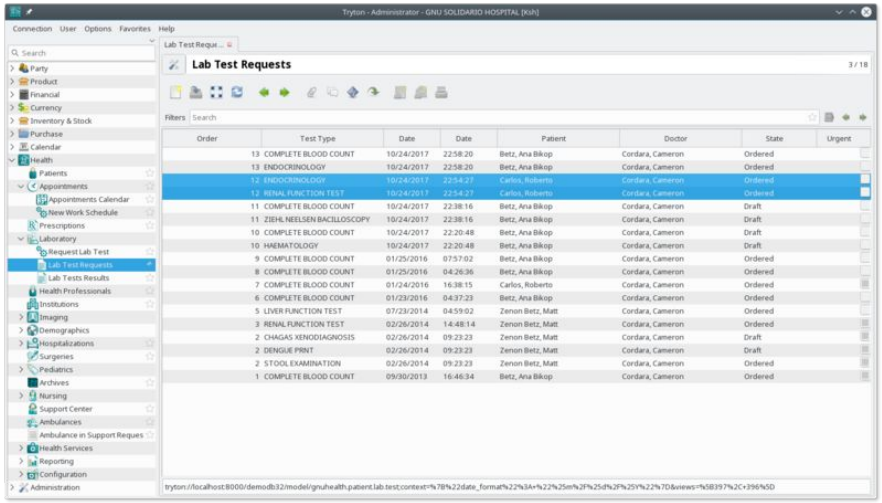




https://2019.igem.org/Team:Bielefeld-CeBiTec/Notebook
https://github.com/bpucker/MybMonday/wiki

Laboratory Information Management System (LIMS)

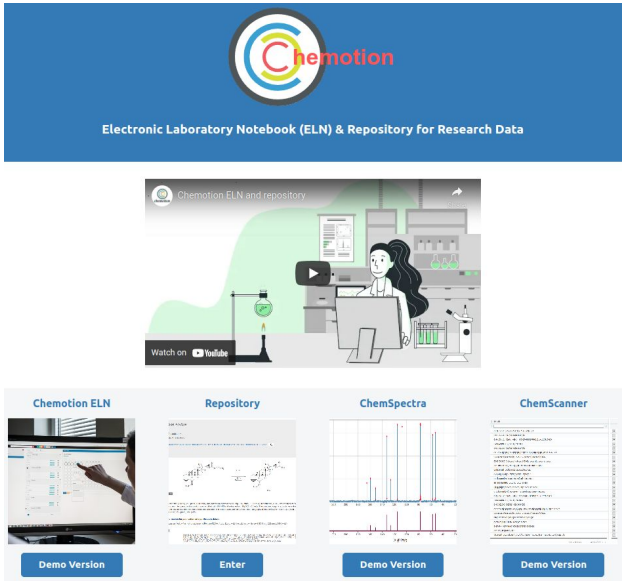
- Workflows are represented and samples are tracked
- Information are linked between analysis
- Different levels of permissions/access
- Examples:
  - Handle a collection of T-DNA insertion lines (GABI-Kat)
  - Manage all samples submitted for sequencing
  - Manage all oligonucleotide orders



https://commons.wikimedia.org/wiki/File:Gnulims_lab_requests.png

Example: Chemotion

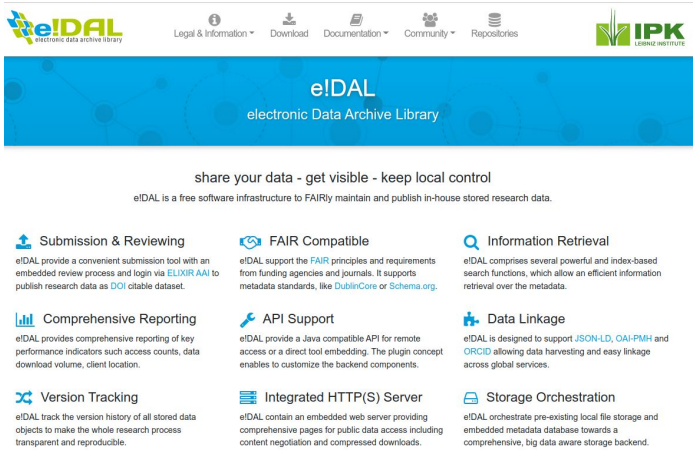
- Research data management tool for chemists
- Electronic Laboratory Notebook (ELN)
- Repository for research data (easy transfer from ELN)
- DOIs are assigned to datasets and protocols



https://www.chemotion.net/chemotionsaurus/index.html; © Chemotion - KIT

Example: e!DAL

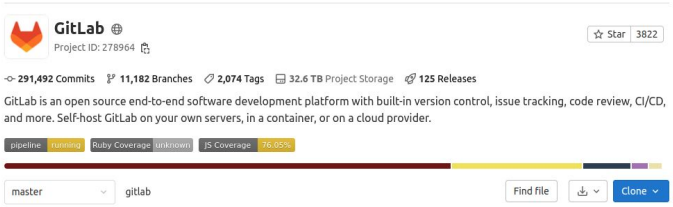
- Maintained at IPK; supported by de.NBI
- DOIs are assigned to submitted data sets
- Reporting about access statistics
- Version tracking
- FAIR compatible and data linkage



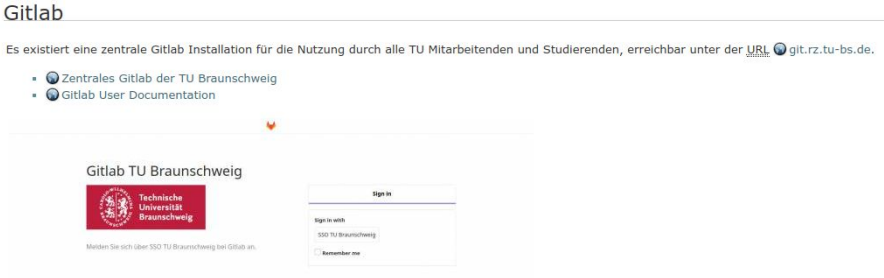
https://edal.ipk-gatersleben.de/

Example: gitlab

- Free version control solution
- Can be used for software development, but also suitable as ELN
- GITZ offers a central service at TUBS: https://doku.rz.tu-bs.de/doku.php?id=server:gitlab
- Non-commercial alternative to github



https://gitlab.com/gitlab-org/gitlab



https://doku.rz.tu-bs.de/doku.php?id=server:gitlab

Example: Benchling

- Cloud-based platform for biotechnology
- Templates for documentations
- Convenient to use
- Compatible with various platforms
- Suitable for certified processes

Lab 4.0: digital lab for higher efficiency

- Connection of lab and analysis processes

- Data from different instruments are synchronized through a cloud
  - Values and pictures are directly inserted into the lab book

- Automation of processes (robotics)
  - Pipetting robot for large scale sample processing
  - Automatic phenotyping facilities

- Samples are labeled with barcodes
  - Barcodes and scanning avoid human errors
  - Can also prevent fraud

Summary: data is everywhere

- Size of data sets is growing
- More sophisticated data collection methods
- Databases enable dissemination/reuse
- Electronic documentation
- Digitalization makes processes more efficient

Course challenge

[add pictures and motivation of specific course challenge here]

Previous knowledge?

- Are there previous reports of similar observations? (internet search)
- Are there publications about possible explanations? (literature search)
- Are there data sets that could be helpful for a study? (re-use)

[Add some screenshots of specific datasets here]

Hypotheses

- Research should be driven by hypothesis (hypothesis testing)
- Hypothesis must be specific and falsifiable
- Proofing hypothesis right is not possible
- Rejecting a hypothesis if possible based on contradicting evidence
- Different hypothesis must not be mutually exclusive

What is your hypothesis?

Example: hypotheses

- Activation of anthocyanin biosynthesis gene *DFR* explains color appearance
- pH change of the soil/water determines the color
- Transcriptional activators of the anthocyanin biosynthesis are triggered
- Competing branches of the flavonoid biosynthesis are inactivated
- Intracellular transport of anthocyanins is changing

What do we need to test a hypothesis?

- Example: activation of anthocyanin biosynthesis gene *DFR* explains color emergence
- Genome sequence (long read sequencing & assembly)
- Structural annotation of the genome sequence (gene prediction)
- Functional annotation of the genes (function prediction)
- Analysis of gene activity (RNA-seq, data re-use)

Disseminate findings and data

- Submit data sets to appropriate repository
- Share documentation and scripts developed for analyses
- Share findings through talks, posters, or publications

Data management tipps

Check the literature

- Was the research question already answered?
- Have others proposed hypotheses to explain the observed phenomenon?
- Reading numerous publications can avoid a lot of unnecessary work

How to find publications?

How to find publications?

- Where can you search?
  - GoogleScholar, PubMed, SciFinder, PubPharm, WebOfScience
  - Various AI-supported search engines (e.g. Bing)
    - Warning: some AIs make up references that do not exist

- What keywords should you use?
  - Try synonyms to find the specific terms people in the field use
  - Try different keyword combinations and orders

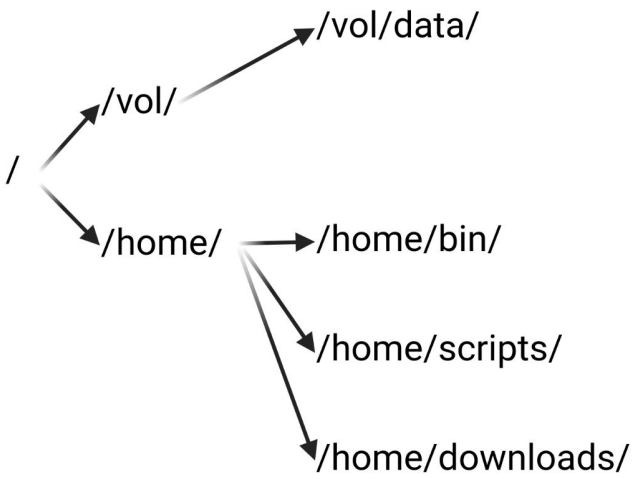
- Which publications should you read?
  - Review articles could be a good start
  - Recent & highly cited articles seem promising
  - Walk through network of citations

How to structure your data?

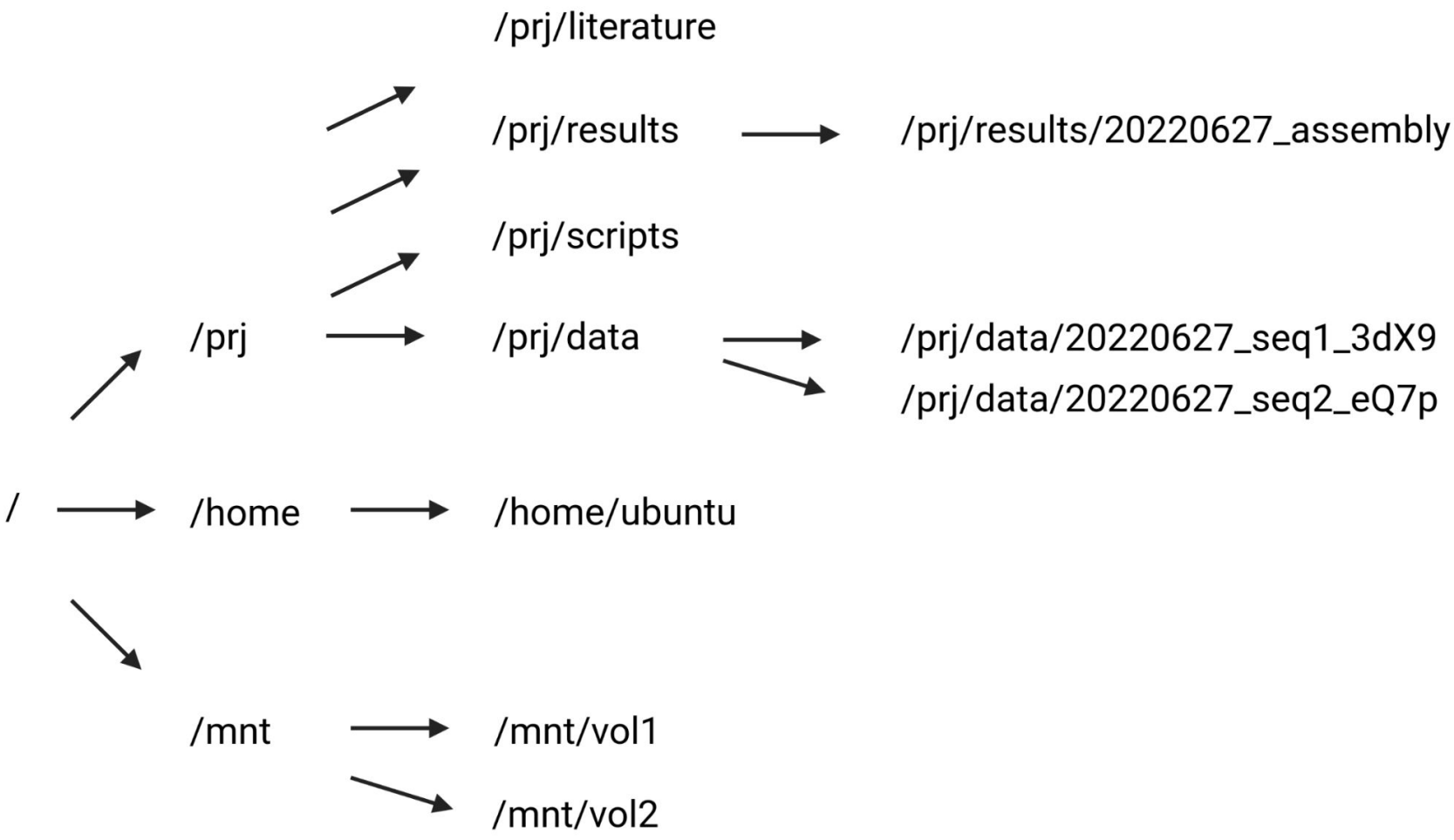
- Document every step (e.g. in a README)
  - Origin of data sets; versions of tools, parameters of analyses
- Keep raw data sets separated from scripts and results
- Sort data by project
- Structure analysis related data sets/results by date

Linux file system structure

- Linux (Ubuntu) is operation system of choice for bioinformatics
- Hierarchical structure of directory (‘/’ is basis)
- Separation of tool installation and data sets



Example: Linux file system



What do you know about file names?

Naming files

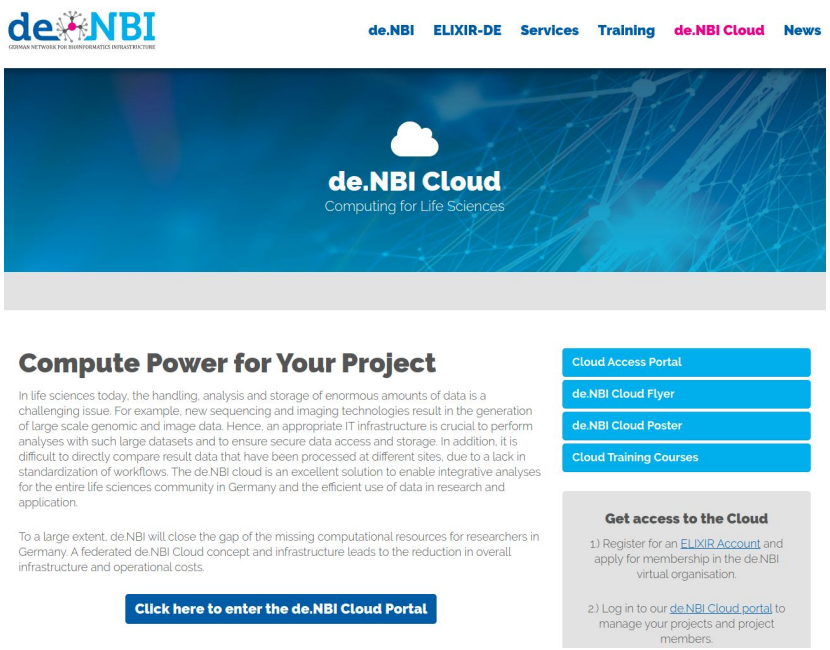
- File names should be informative
- Never use ‘new‘ or ‘final’; use version numbers instead
- Use date as file name prefix (e.g. 2022-06-27 or 20220627)
- Never use spaces in file or folder names (underscore or minus as replacement)
- Include your initials as suffix in collaborative projects

Documentation

- Document as much as possible
- Others must be able to repeat your experiments/analyses
- Document dates of data acquisition and processing
- Document all steps of data processing
- Document versions and parameters of applied tools

de.NBI cloud

- Virtual machine (VM) for data analysis
- Accounts are required for access (ORCID or TUBS SSO for login)
- Addition to project required for access
- User create pair of private and public keys for authentication



Transferring files

- Filezilla: graphical user interface for file transfer protocols
  - https://filezilla-project.org/
- Scp (secure copy): command line file transfer method
- Wget: command line file transfer method
  - https://ftp.gnu.org/old-gnu/Manuals/wget-1.8.1/html_mono/wget.html
- Rsync: sophisticated file transfer method that avoids redundant transfers
  - https://wiki.ubuntuusers.de/rsync/

Permissions

- Files can have different permissions:
  - Read (r)
  - Write (w)
  - Execute (x)

- Users have full permissions to edit their own files

- Downloaded files are usually not executable without adjustment

- chmod XXX <FILE_NAME> … can be run to change file permissions

Linux introduction (1)

Connection to virtual machine (VM):

```
$ ssh -i /path/to/private_key ubuntu@123.133.7.49 -p 1234
(base) ubuntu@agilezuse-10552:~$
```

'$' is used to indicate that following text needs to go into terminal

'#' indicates comment (should not be transferred into terminal)

Frequent issues:

1) Path to private key file not correct
2) Private key file too public

Linux introduction (2)

- Moving through the folder structure:
  - $ cd /full/path/to/folder #change into specific folder
  - $ cd subfolder #change into subfolder
  - $ cd .. #change into parent directory

- Checking content of a folder:
  - $ ls #shows content of current folder
  - $ ls -lh #shows more details
  - -l triggers display of additional details
  - -h human readable
  - -a show also hidden files

How to install bioinformatics tools

- Install tools from package management (apt-get install)

Installing mafft package on Ubuntu is as easy as running the following command on terminal:

```
sudo apt-get update
sudo apt-get install mafft
```

- Download a precompiled binary

Releases

The **kallisto** GitHub repository is here.

Version	Date					
Release notes: v0.46.1	October 04, 2019	Mac	Linux	Windows	Rock64	Source
Release notes: v0.46.0	June 12, 2019	Mac	Linux	Windows	Rock64	Source
Release notes: v0.45.0	November 17, 2018	Mac	Linux	Windows	Rock64	Source
Release notes: v0.44.0	January 29, 2018	Mac	Linux	Windows	Rock64	Source

- Compile from source

COMPILING FROM SOURCE

Download the latest release from and uncompress it

```
# Get latest STAR source from releases
wget https://github.com/alexdobin/STAR/archive/2.7.10b.tar.gz
tar -xzf 2.7.10b.tar.gz
cd STAR-2.7.10b

# Alternatively, get STAR source using git
git clone https://github.com/alexdobin/STAR.git
```

Compile under Linux

```
# Compile
cd STAR/source
make STAR
```

For processors that do not support AVX extensions, specify the target SIMD architecture, e.g.

```
make STAR CXXFLAGS_SIMD=sse
```

- Install via conda

Installers

linux-64 v0.11.1

osx-64 v0.6.0

conda install

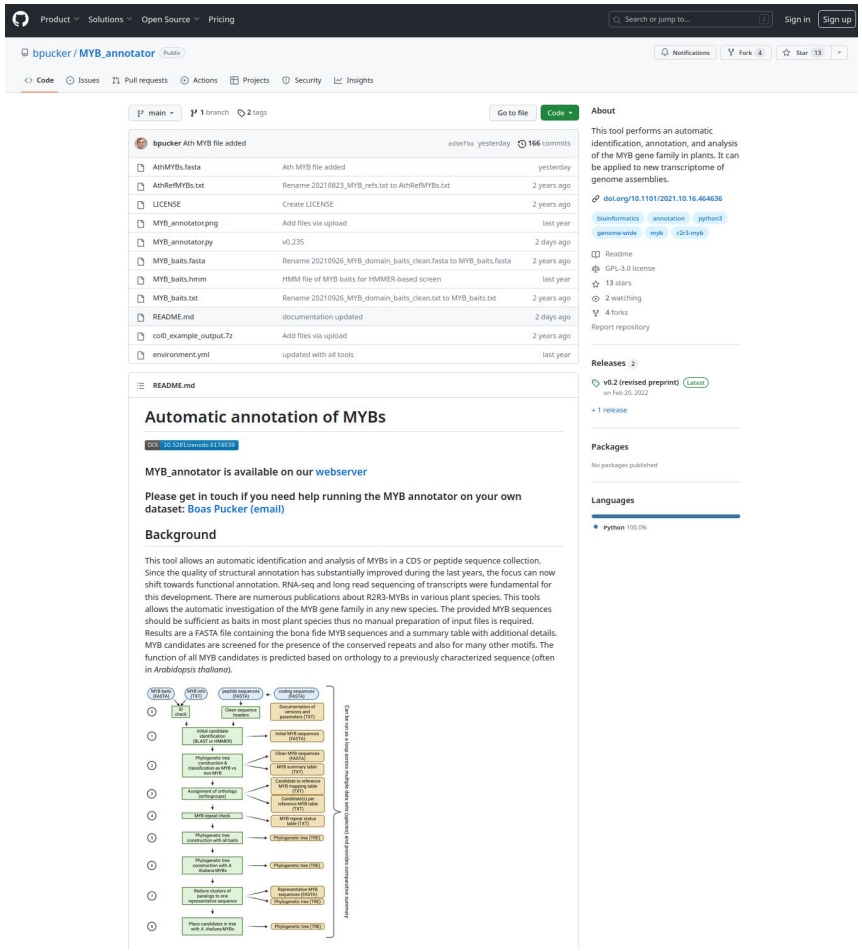
To install this package run one of the following:

```
conda install -c bioconda shasta
```

https://howtoinstall.co/en/mafft
http://pachterlab.github.io/kallisto/download
https://github.com/alexdobin/STAR
https://anaconda.org/bioconda/shasta

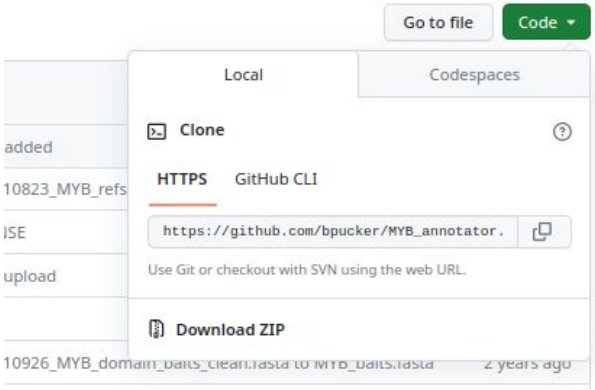
Retrieving tools from GitHub

- Find GitHub repository corresponding to tool of interest
- Read the installation instructions
- Download single file via “Raw”, “Save as”
- Download everything via “Code” as ZIP archive
- Use git clone to get the content



Cloning a GitHub repository

1. Go to GitHub repository website
2. Click “code”
3. Copy URL (HTTPS)
4. Open a terminal
5. Change working directory to the location where you like to have the tool
6. Clone the repository: `git clone <URL>`



https://docs.github.com/en/repositories/creating-and-managing-repositories/cloning-a-repository

Virtual environments

- Python tool (venv) for dependency management and project isolation
- Conda keeps packages separated and works without administrator rights
- Increase reproducibility of research
- Configure virtual environment:
  - python3 -m pip install --user --upgrade pip
  - python3 -m pip --version
  - python3 -m pip install --user virtualenv
  - python3 -m venv env
  - source env/bin/activate
  - [here you can do things inside the virtual environment]
  - deactivate #to leave the virtual environment again

https://docs.python.org/3/library/venv.html

Running Python scripts

- Run script or open argument infos:
  - $ python <SCRIPT_NAME>
- Run script with arguments:
  - `$ python <SCRIPT_NAME> --argument1_name <ARGUMENT1> --argument2_name— <ARGUMENT2>`
- Scripts show help message if started with insufficient arguments

```
python3 ./KIPEs3.py --baits ./flavonoid_baits/ --positions ./flavonoid_residues/ --out ./ --subject ./croton_red.fasta --seqtype pep --scoreratio 0.3 --simcut 40.0 --minsim 0.4 --minres 0.0 --minreg 0.0 --possibilities 3 --cpus 1
```

Running other tools

- Show help message:
  - $ <NAME_OF_TOOL>
  - $ <NAME_OF_TOOL> -h
  - $ <NAME_OF_TOOL> -- help

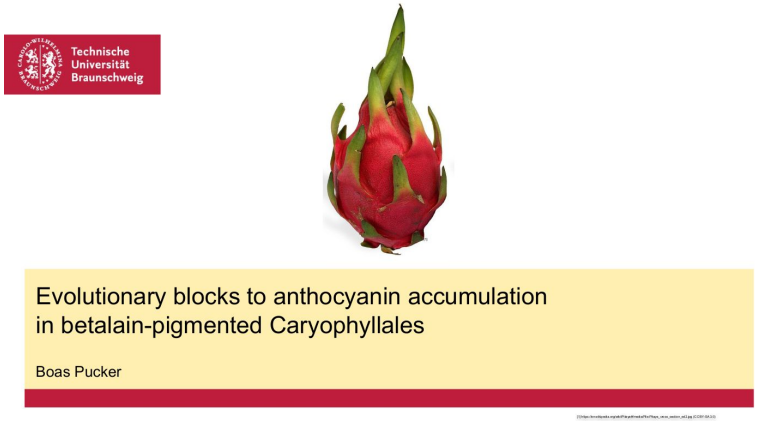
- Providing arguments is different for each tool:

- Most tools show help message if provided with insufficient/wrong arguments

Giving presentations

Presentation structure

- Title slide with picture
- Problem/challenge
- Results
- Summary & conclusions
- Future directions
- Acknowledgements



Story telling

- Introduction should be easy to follow for non-experts
- Clearly present motivation
- Questions can catch attention and provide structure
- Connect different elements of the presentation

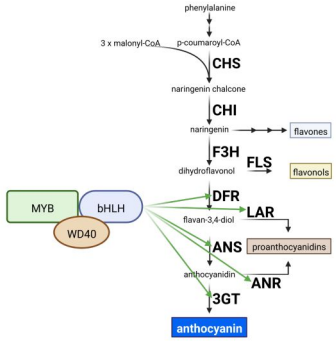
Why are there no anthocyanins in betalain-pigmented species?

Clear slides

- Reduce text
- Replace text with figures
- One clear message per slide
- Large font size

Contribution of transcription factors

- MYBs and bHLHs are two of the largest transcription factor families in plants
- Specific MYBs and bHLHs form complexes to regulated flavonoid biosynthesis genes
- Different complexes regulate anthocyanin/proanthocyanidin biosynthesis



Tools for presentation creation

- PowerPoint
- GoogleSlides (“free”)
- LaTeX (e.g. overleaf)
- Templafy
- Prezi

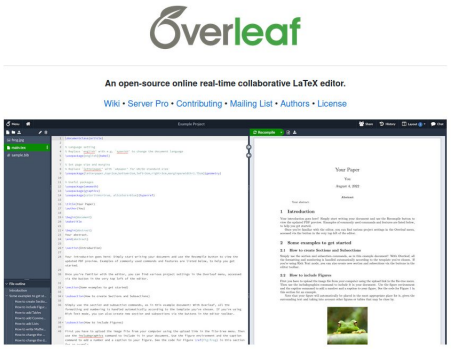


Figure 1: A screenshot of a project being edited in Overleaf Community Edition.

https://github.com/overleaf/overleaf

Tools for figure generation

- PowerPoint/GoogleSlides
- bioRender
- InkScape

Social media/networks

Why are scientists using social media?

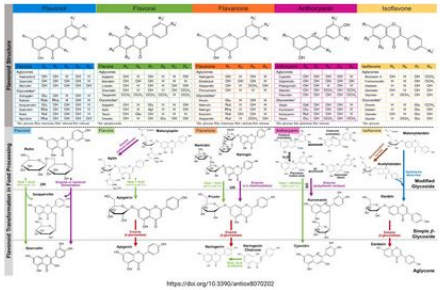
- Scientists share publications, open positions, and other news
- Important platform for science communication
- Platform for scientific discussions
- Learn about latest publications and trends

SciComm posts

- Statements need to be scientifically correct
- Explanations need to be simple
- Connect information to emotion (cute pictures)
- Engage in discussions

(42) Biochemical diversity of flavonoids

> ***TWEET:*** Biochemical diversity of flavonoids is based on hydroxylation, glycosylation, methylation, acylation, and many other modifications #FlavonoidFriday (details: https://bit.ly/3dKUQnN)

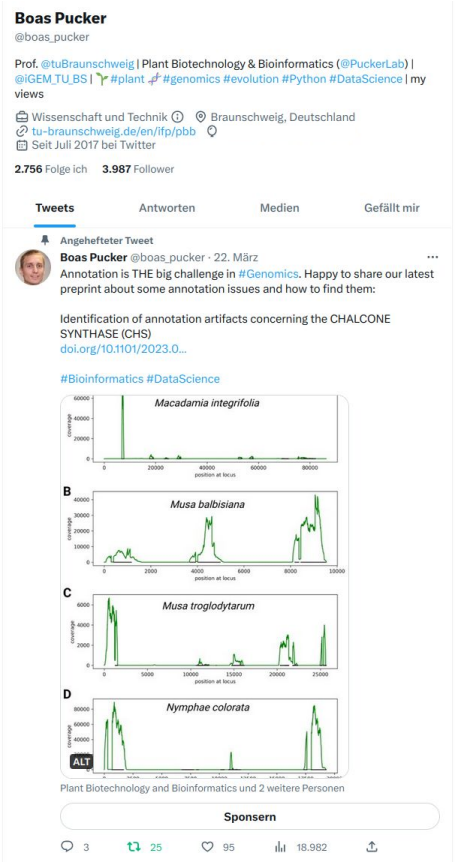


Hydroxylation patterns and other modifications of the aglcyon distinguish between different types of flavonoids. Aglycons can be modified through the successive addition of sugar moieties. The 3 and 5 position of the C ring are most frequently modified. Different sugar groups and can be attached by a large set of different glycosyltransferases as recently reviewed by Tohge et al., 2017.

https://github.com/bpucker/FlavonoidFriday

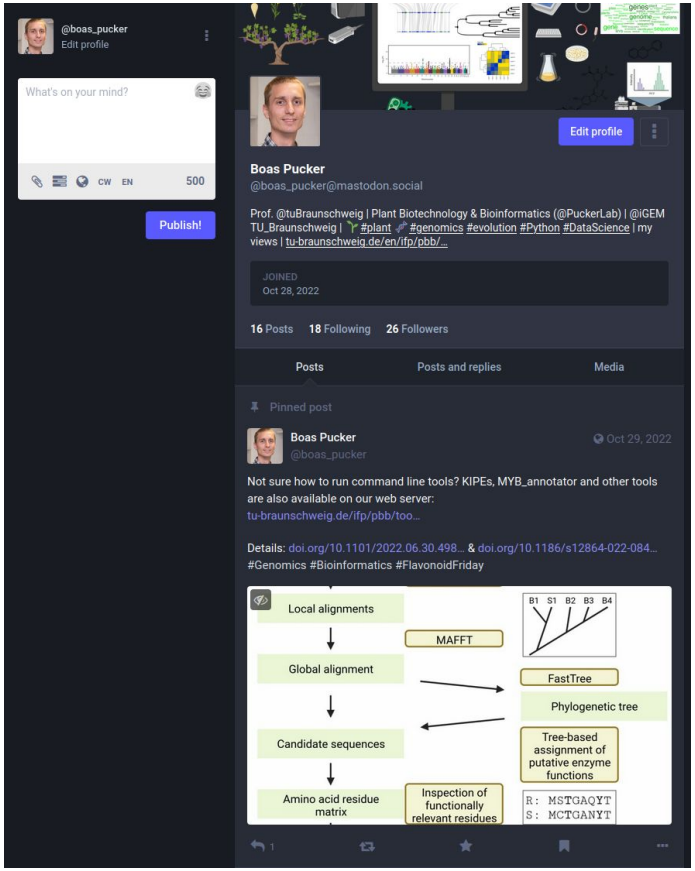
Twitter

- Important platform for science communication
- Scientists share publications, open positions, and other news
- Length of messages is limited (280 characters)
- Links will trigger generation of preview
- Pictures and videos can boost views
- Hashtags can be used as key words (avoid excessive use)



Mastodon

- Similar to Twitter and presented as replacement
- User own their data
- Running on multiple different servers
- Messages can be 500 characters

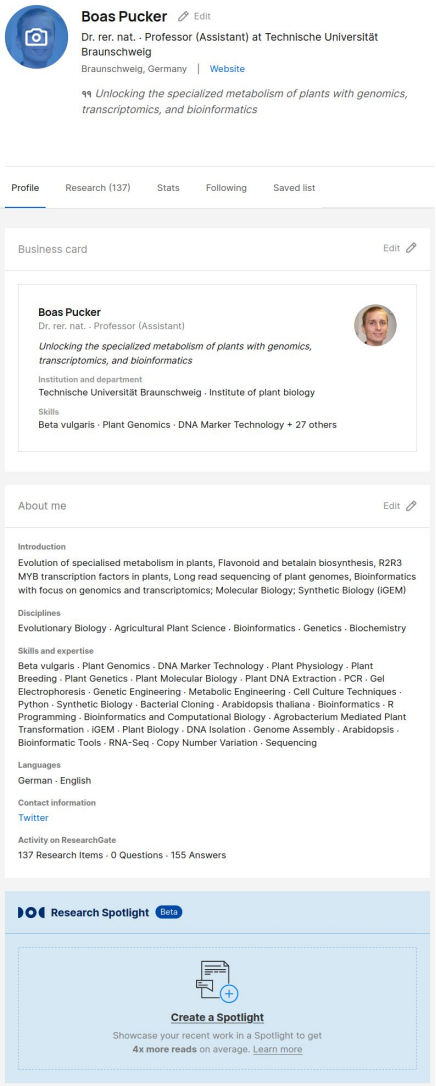


Instagram

- Excellent for sharing pictures and videos
- Communicating science to public
- Dedicated to young audience

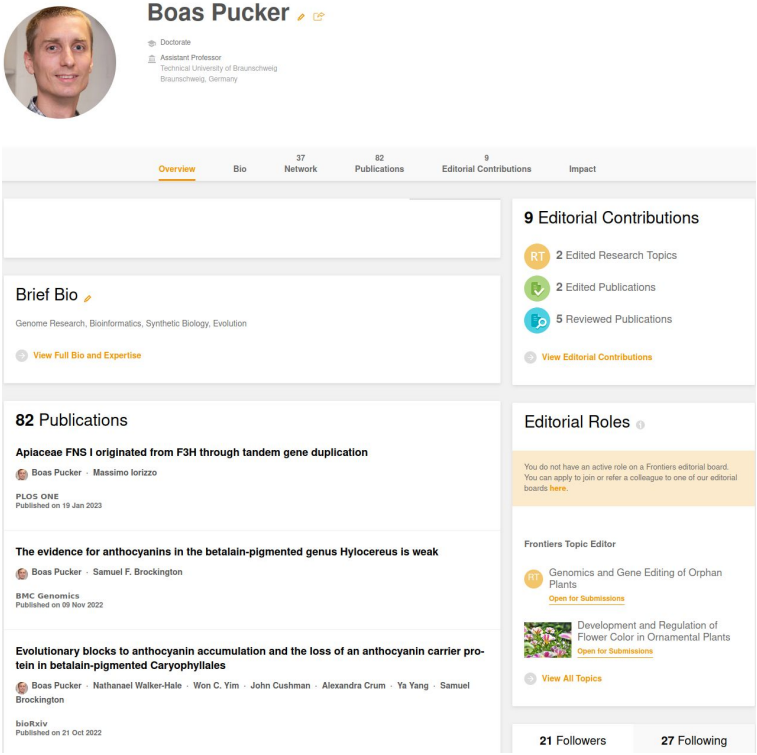
ResearchGate

- Sharing full texts of publications (not always permitted by publisher)
- Promoting publications
- Presenting research interests
- Advertising open positions
- Option for questions and discussions



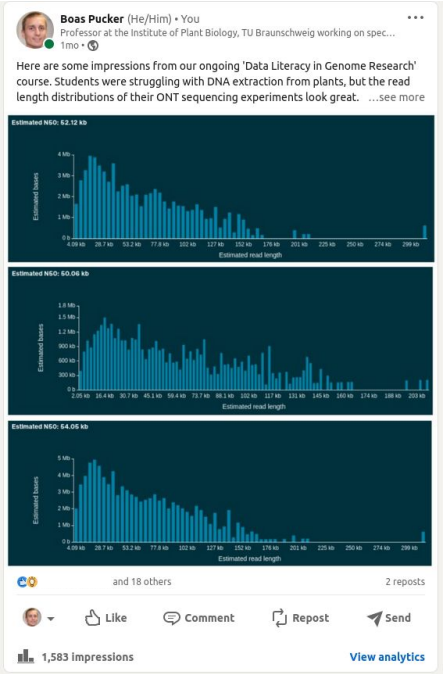
Loop (Frontiers)

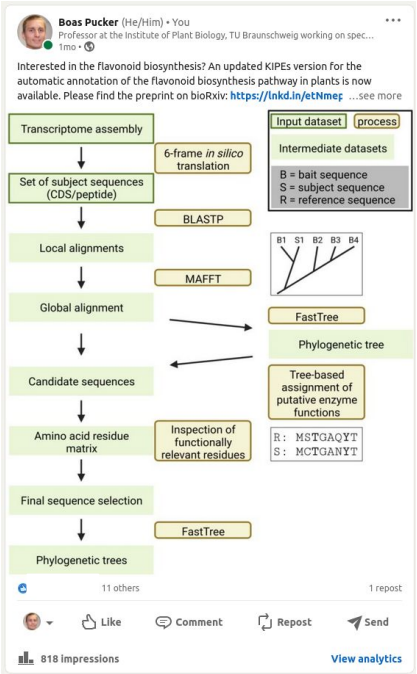
- Social network platform operated by Frontiers
- Handles review process at Frontiers journals
- Journals try to catch researchers and to collect information?



LinkedIN

- Important for exchange with industry
  - Students can leave academia
  - Scientists can find funding options
- Excellent platform to learn about job opportunities
- Scientists share publications and other news

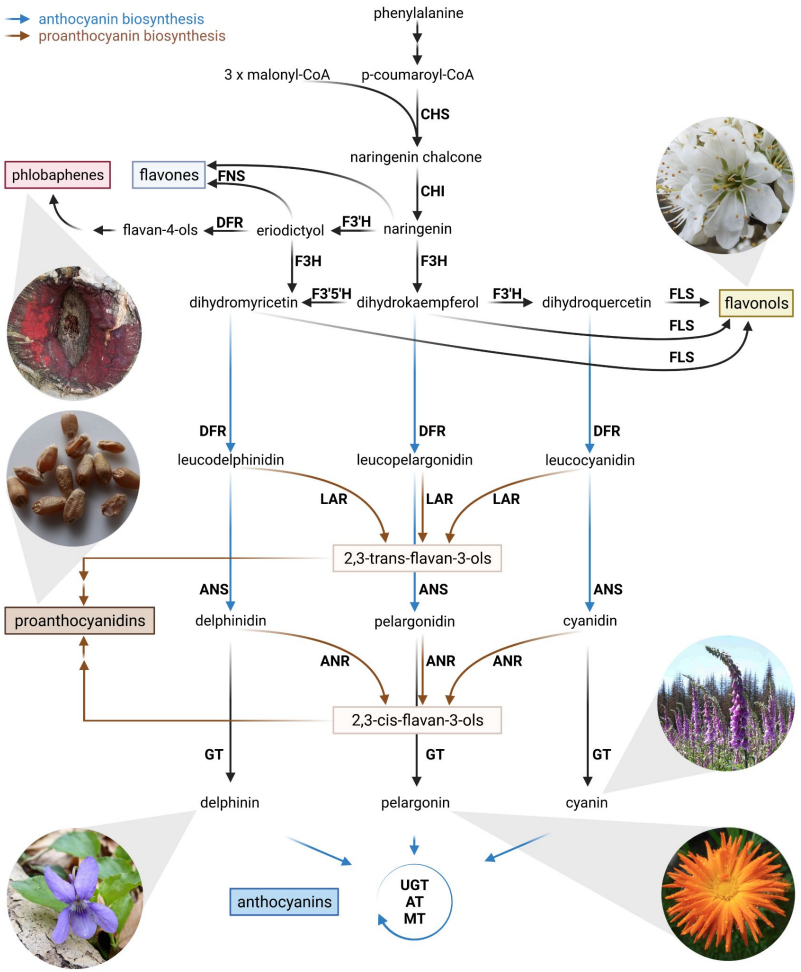




Flavonoid biosynthesis

Flavonoid biosynthesis

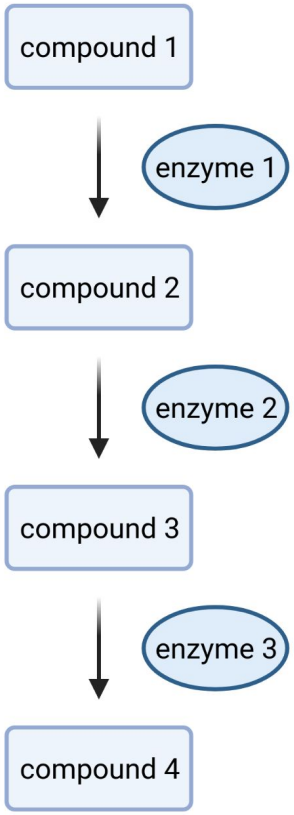
- Phenylalanine is common substrate
- Flavones, flavonols, phlobaphenes, proanthocyanidins, anthocyanins are the major groups
- enzymes catalyze different reactions: flavonoid 3'-hydroxylase (F3'H), flavonol synthase (FLS), dihydroflavonol 4-reductase (DFR), …



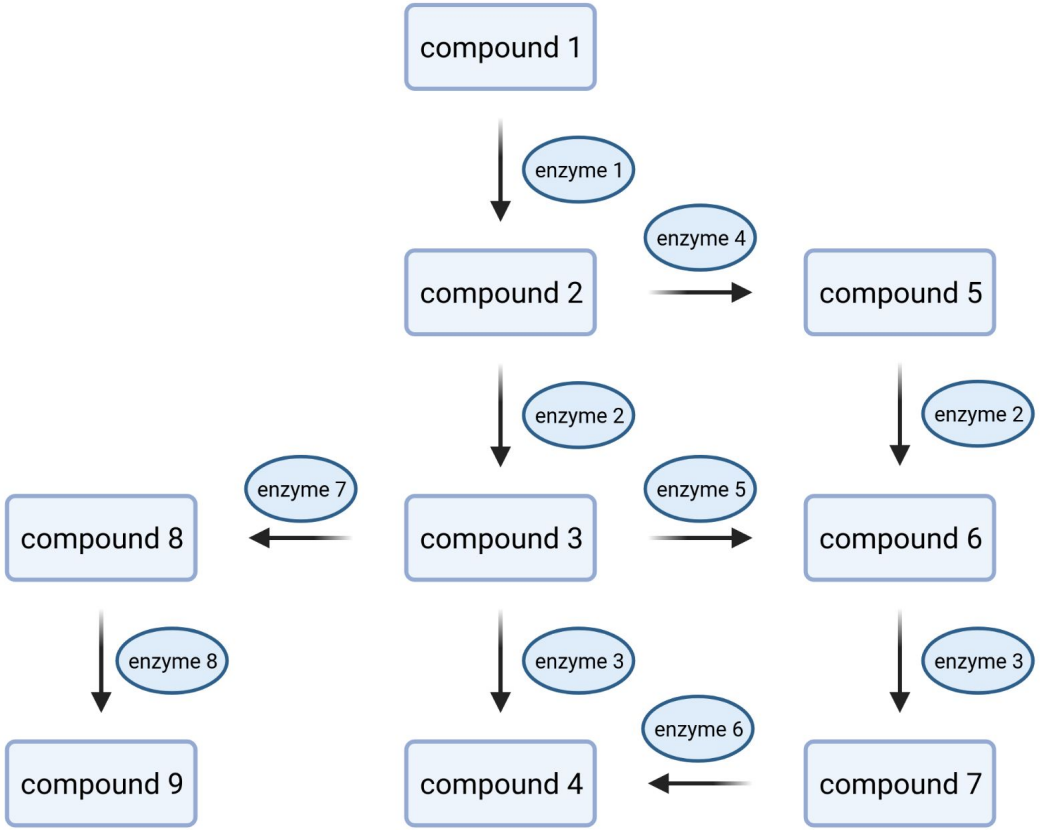
Pucker & Selmar, 2022: 10.3390/plants11070963

Pathways are networks

Text book

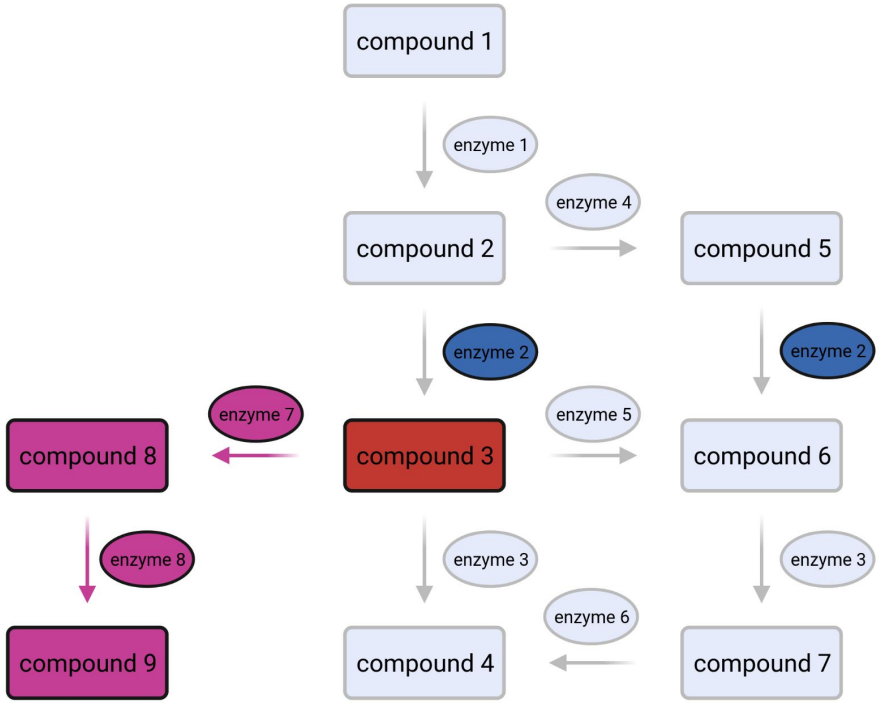


Reality



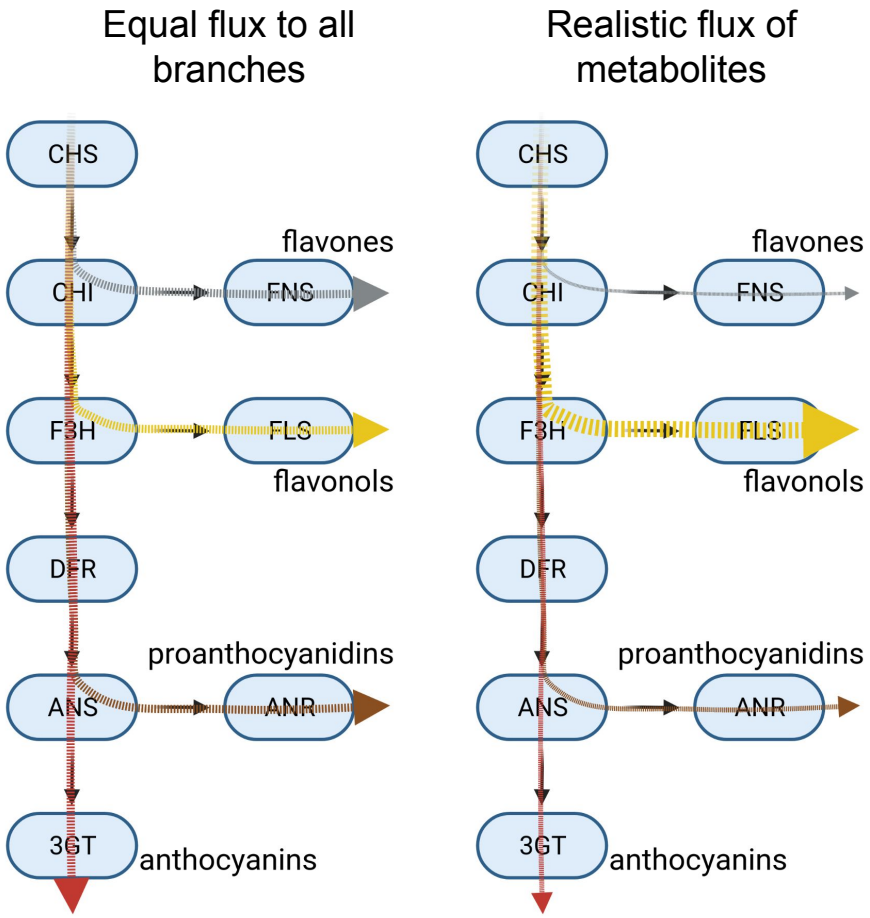
Metabolic networks

- Metabolic networks are characterized by hubs (central intermediates)
- Many enzymes show promiscuity i.e. catalyze different reactions
- Branches could be considered linear pathways



Competing branches

- Different branches of a pathway can compete for substrate
- Activity of different branches can vary between conditions/tissues
- Metabolic modeling can be applied to optimize flux through pathway

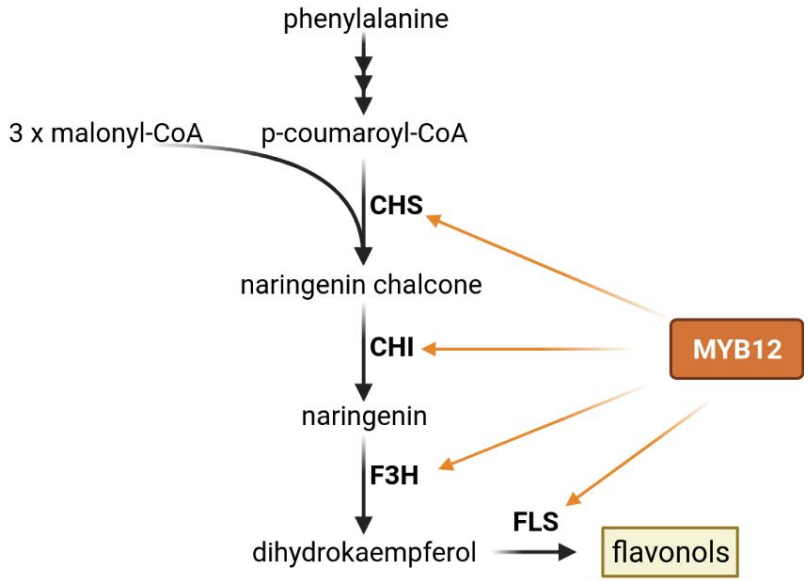


Model system - pathway

- Easily detectable product
- Stable intermediates that can be quantified
- Gene knock-outs must not be lethal
- Conserved across many plant species
- Community working on the pathway
- Different branches
- …

Flavonols - Coexpression of pathway genes

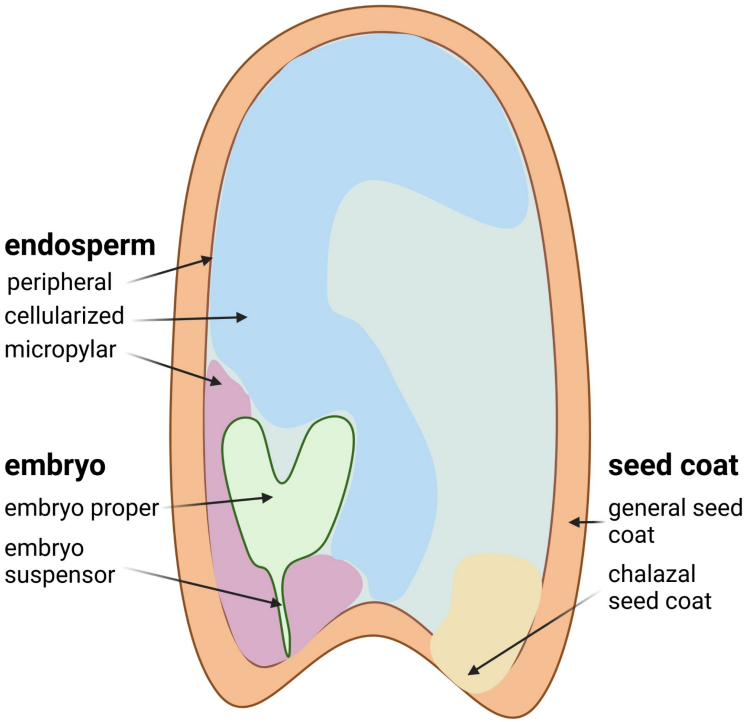
- Shared transcription factor can explain similar expression patterns
- Example: MYB12 controls the flavonol biosynthesis through activation of *CHS*, *CHI*, *F3H*, and *FLS*
- Expectation: flavonol biosynthesis genes *CHS*, *CHI*, *F3H*, and *FLS* should show a similar expression pattern



Stracke *et al.*, 2007/2010/2014

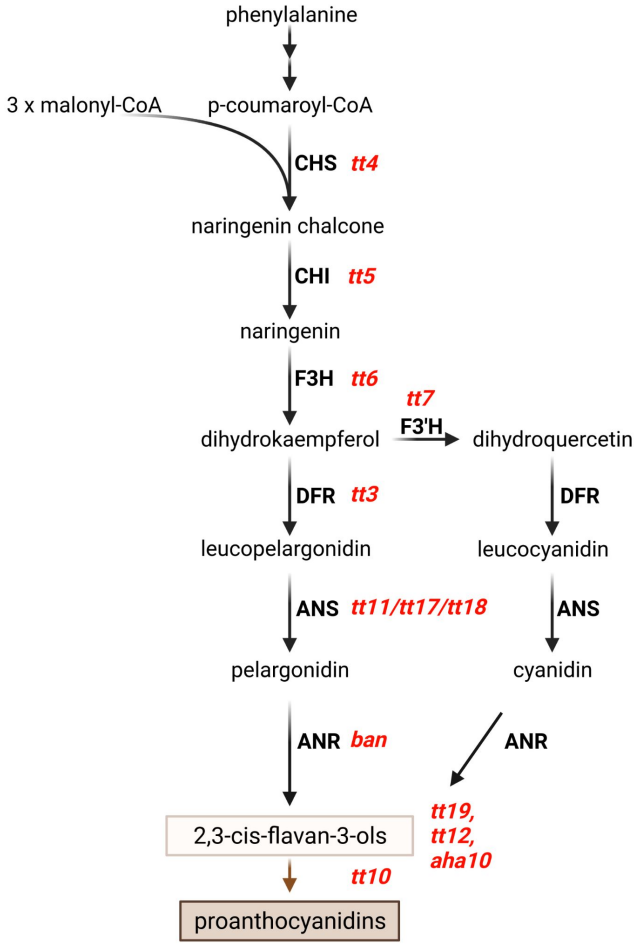
Proanthocyanidins - Transparent testa

- Brown proanthocyanidins are located in the testa
- Transparent testa = lack of proanthocyanidins (no pigmentation)



Transparent testa mutants

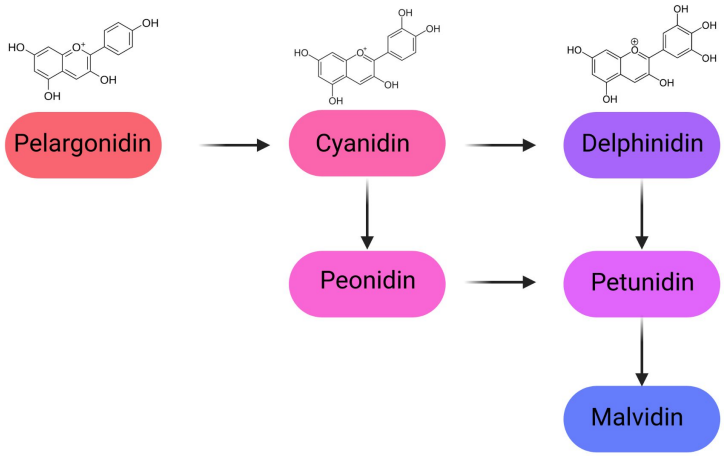
- Large collection of different tt mutants
- Numbering is based on order of discovery
- Checking if new *transparent testa* (*tt*) mutants are allelic with existing ones



Appelhagen et al., 2014: 10.1007/s00425-014-2088-0

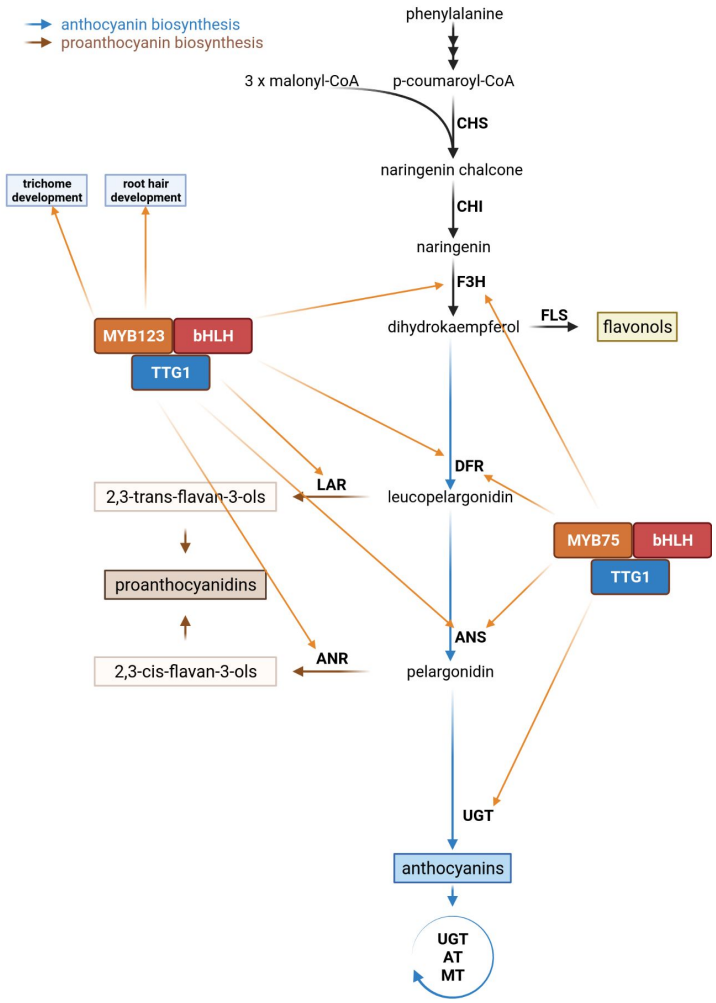
Anthocyanin loss

- Anthocyanins are responsible for red to blue pigmentation of flowers
- Anthocyanins are derivatives from phenylalanine through the flavonoid biosynthesis
- *Digitalis purpurea* is an ornamental plant with biomedical potential
- Different varieties of *D. purpurea* show pigmentation differences



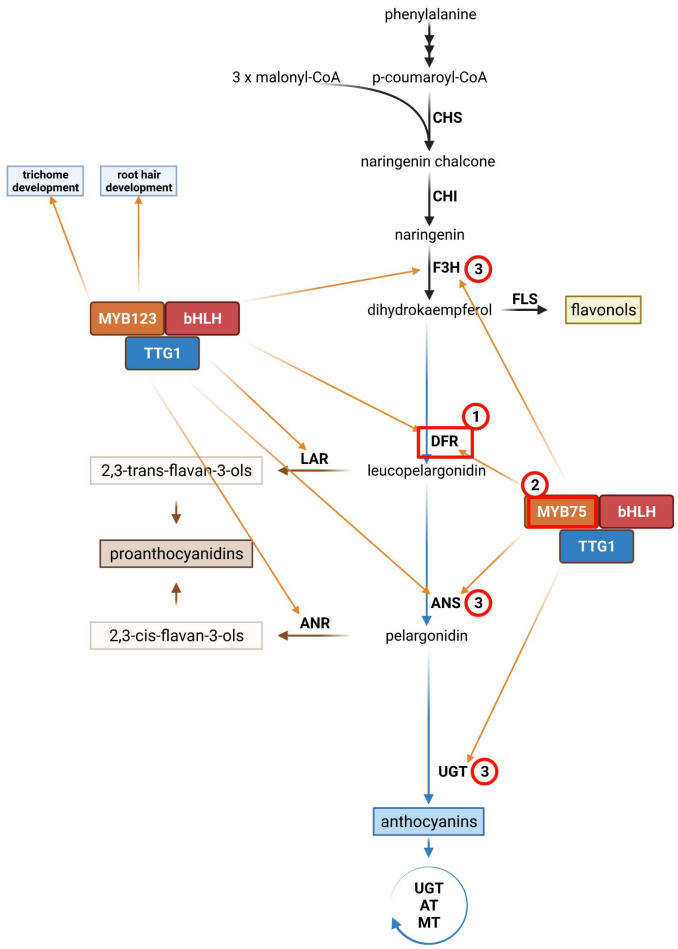
MBW complex: MYB+bHLH+WD40

- Genes can be regulated by multiple TFs (e.g. *DFR* by MYB123 and MYB75)
- TFs can control different processes (e.g. proanthocyanidins, trichome development, root hair development)
- Co-expression of TFs and structural genes in pathways is not perfect



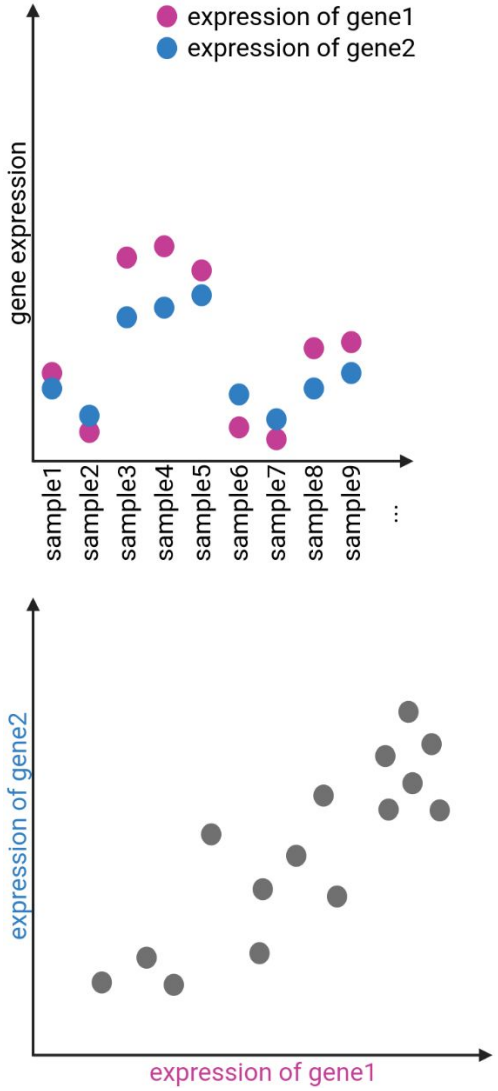
Revealing pathways through coexpression analysis

- Bait genes are previously characterized genes with a function of interest e.g. encode an enzyme in the same biosynthesis pathway
- Shared transcription factors of a pathway can be helpful to identify all structural genes of a pathway
- Knowledge from other species can be applied in this step (details in later section)



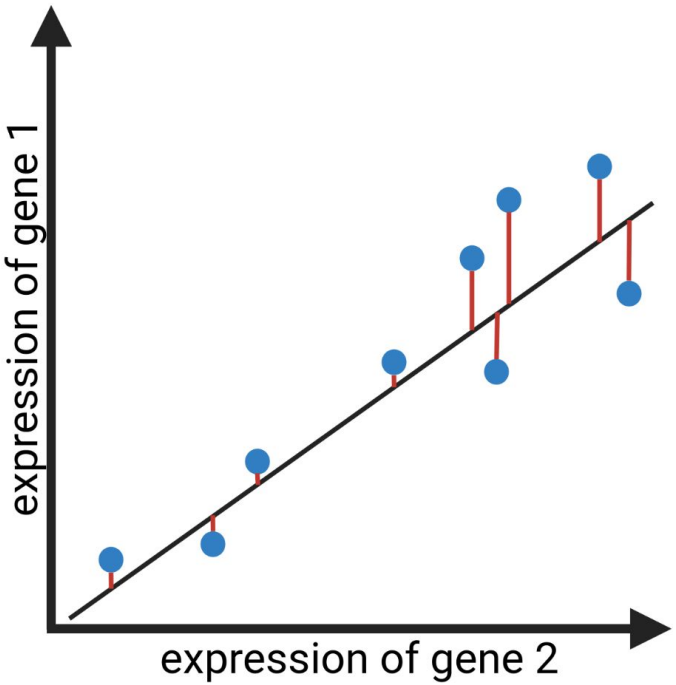
Concept of coexpression

- Genes can show similar expression values across numerous samples
- Reality usually results in similar, but not identical patterns
- Different samples could be different plant parts of plants cultivated under different conditions



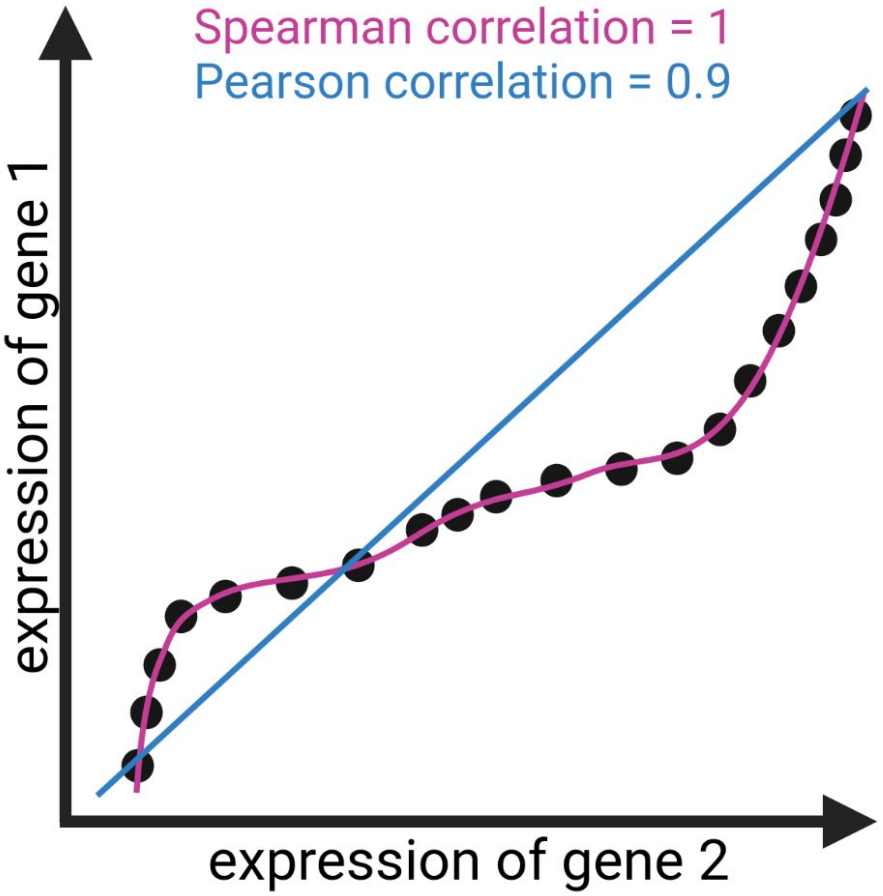
Pearson correlation coefficient

- Line is fitted to achieve minimal distance of all data points to the line
- Only good for linear correlation

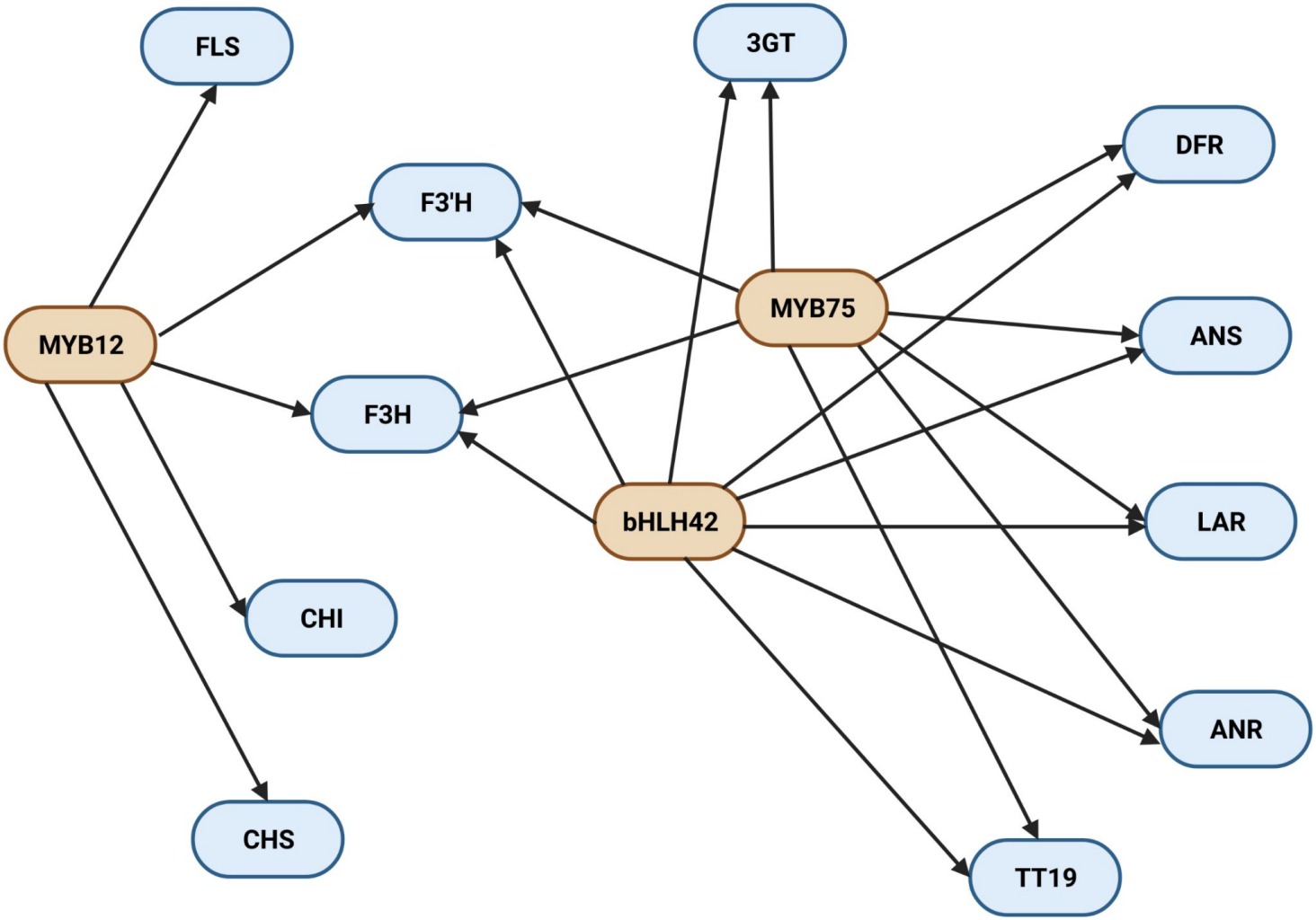


Spearman correlation coefficient

- Rank-based correlation coefficient
- Not restricted to linear correlation
- More appropriate for gene expression which might not show linear correlation



Coexpression network example



Summary

- Importance of data literacy
- Electronic lab books & repositories
- Hypothesis-driven research
- Hands-on data management tipps
- Linux introduction
- Giving presentations
- Social media/networks
- Flavonoid biosynthesis and its regulation

Time for questions!

Questions

1. What do you know about the growth of (sequence) databases?
2. What is Data Literacy?
3. Why is Data Literacy important?
4. Which properties has a hypothesis?
5. What are important considerations when naming files?
6. What needs to be documented?
7. What are advantages of an ELN?
8. What is LIMS?
9. What characterizes lab 4.0?
10. What is a good structure for a scientific talk?
11. Which social media have a relevance for scientists?
12. Which factors can cause the lack of anthocyanins?
13. Which branches belong to the flavonoid biosynthesis?
14. What is the MBW complex?