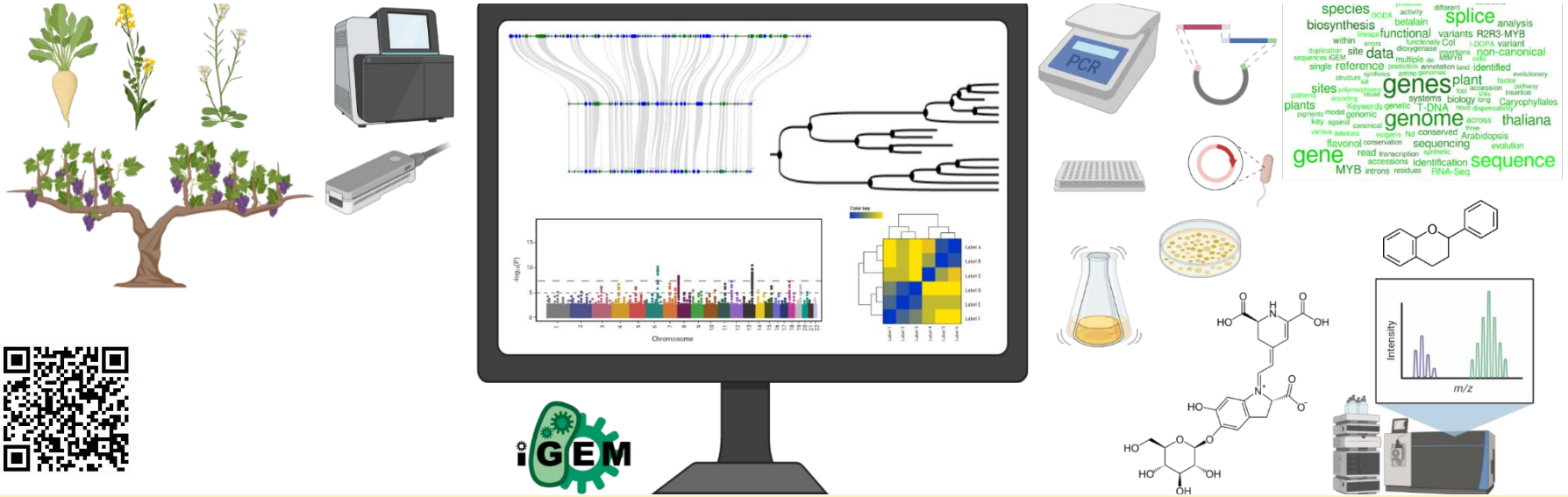
Technische Universität Braunschweig



Utilization of RNA-seq data

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

Availability of slides

- All materials are freely available (CC BY) - after the lectures:
  - StudIP: **Applied Plant Transcriptomics**
  - GitHub: https://github.com/bpucker/teaching

- Questions: Feel free to ask at any time

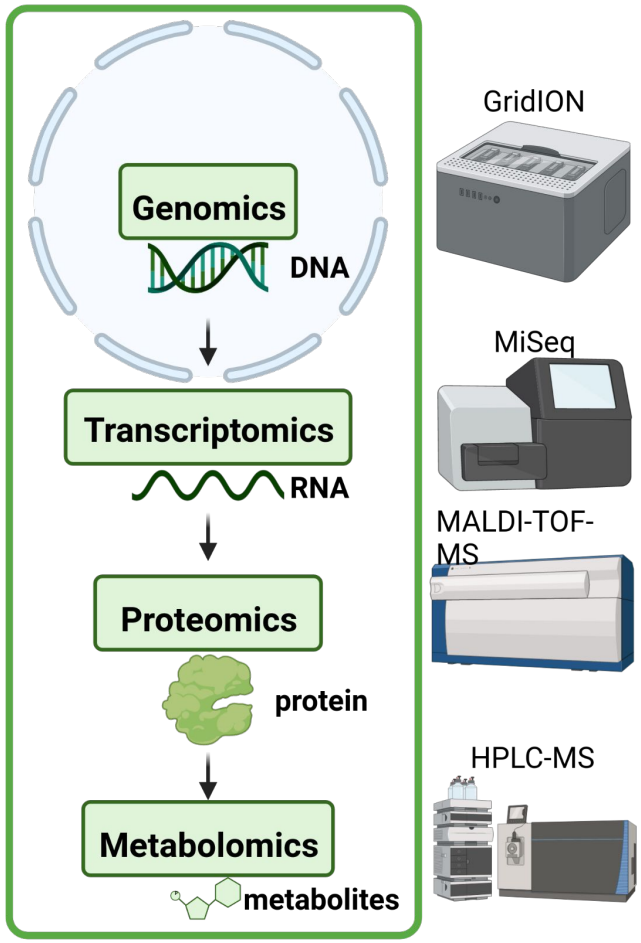
- Feedback, comments, or questions: b.pucker[a]tu-bs.de

Overview

- Gene prediction
- Coexpression and coexpression networks
- Neo- and subfunctionalization
- Mapping-by-sequencing
- Cross-species transcriptomics

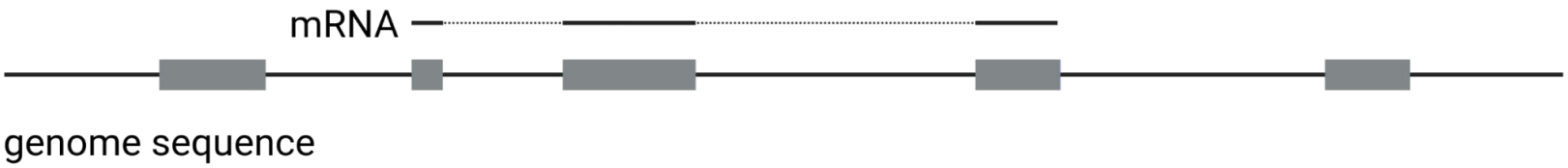
Transcriptomics in context

- Transcriptomics is most powerful in the context of other omics data sets
- Genomics and transcriptomics benefit from molecules with uniform properties
- Proteomics and metabolomics involve more chemically diverse molecules thus comprehensive quantification is not feasible



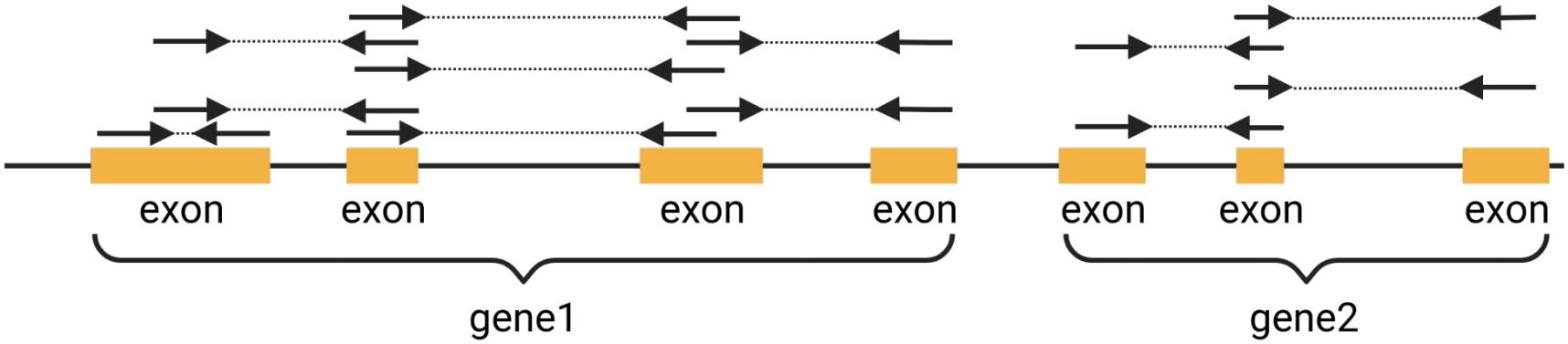
De novo transcriptome assembly - summary

- Cost-effective way to represent the most important parts of a genome
- Computational costs are substantially lower than for a genome sequence assembly
- Reveals information about ‘active’ genes
- Can provide hints for the annotation of a genome sequence



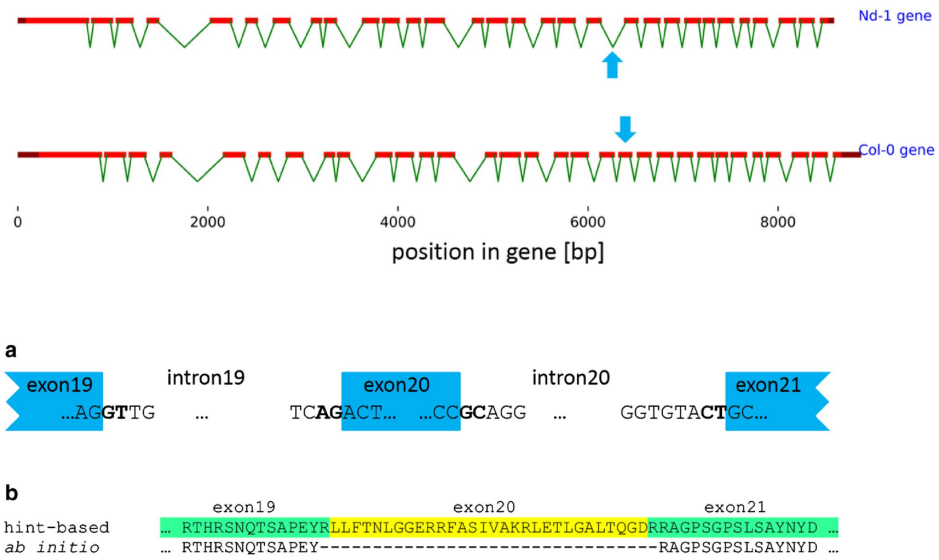
Hints for gene prediction

- RNA-seq reads can inform about positions of transcribed parts in a genome genes
- Positions of introns can be inferred from alignment gaps
- Connection of exons can be inferred from split read alignments
- Stranded RNA-seq data sets are best for gene prediction



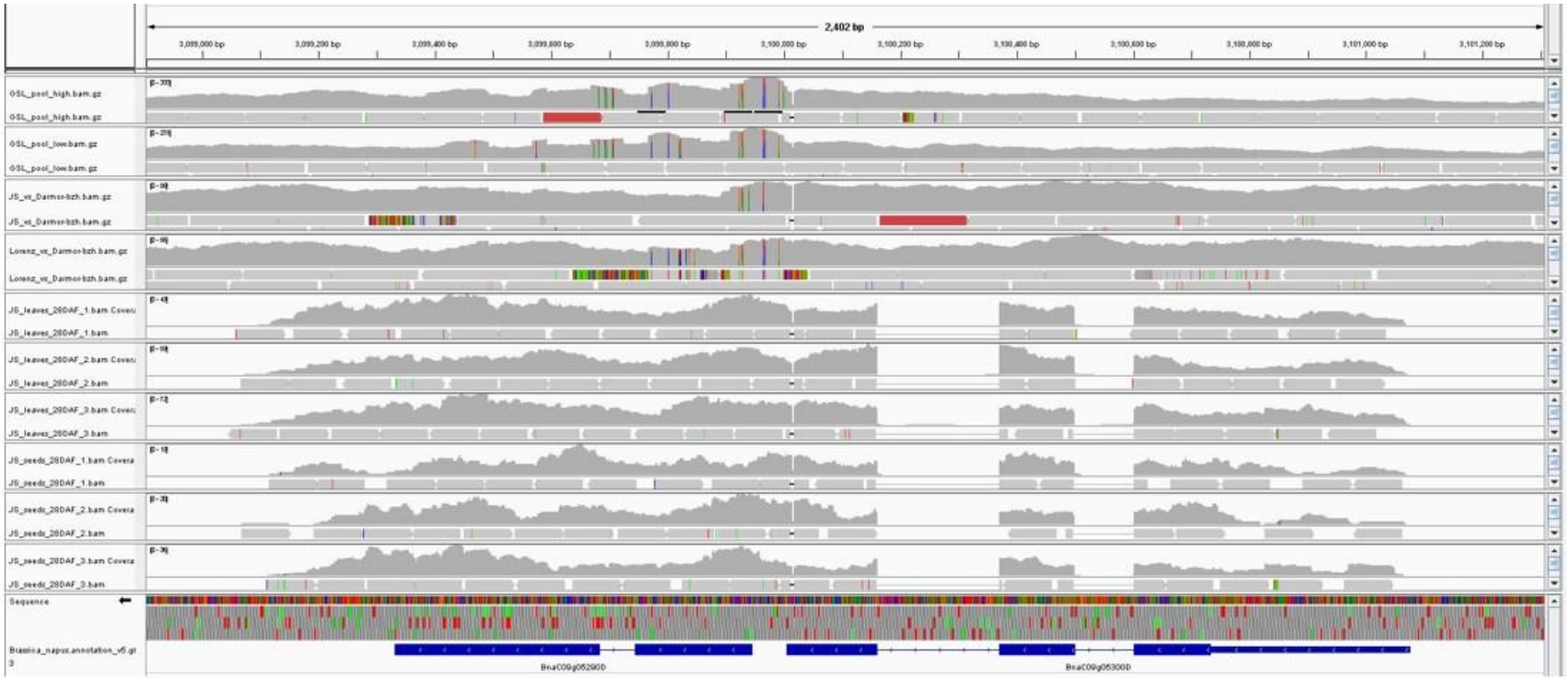
Annotation of non-canonical splice sites requires hints

- Canonical splice sites: GT…AG
- Major non-canonical splice sites: GC…AG, AT…AC
- Minor non-canonical splice sites: NN…NN



https://doi.org/10.1186/s13104-017-2985-y

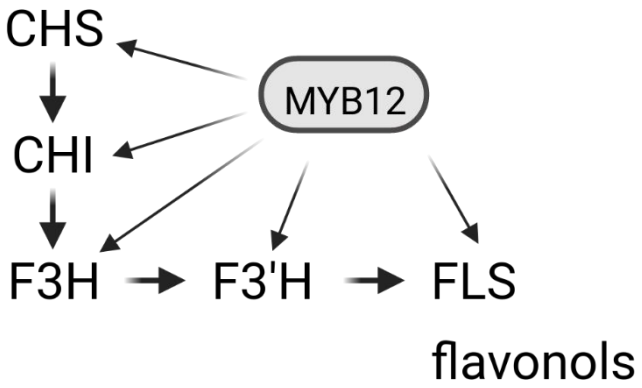
Finding non-functional alleles

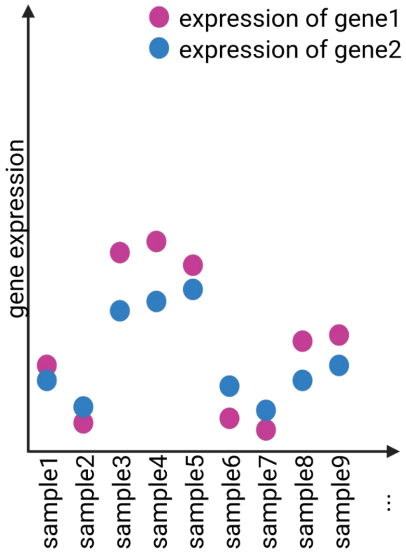


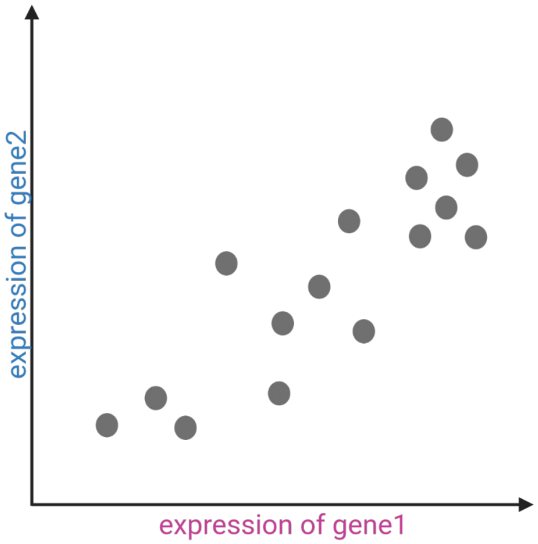
https://doi.org/10.3390/genes13071131

Co-expression analysis

- Genes of the same biosynthesis pathway show similar expression patterns
- Co-expression is a very powerful indication for connection between genes



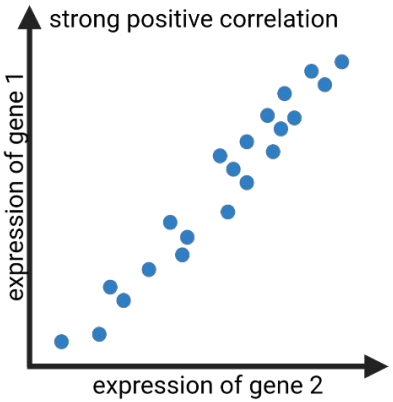


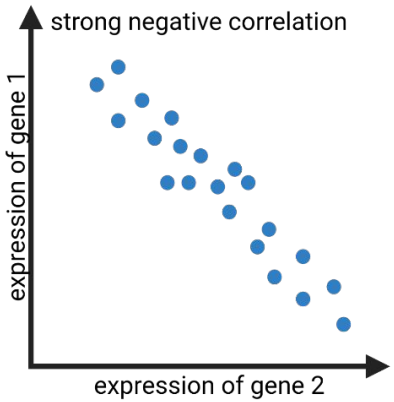


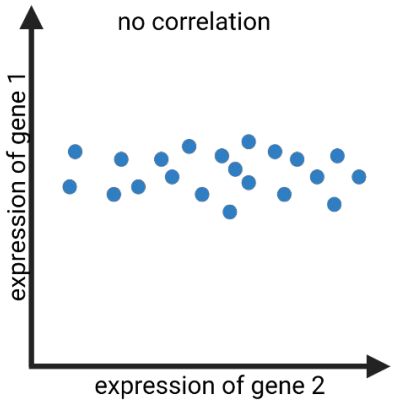
Correlation vs. causation

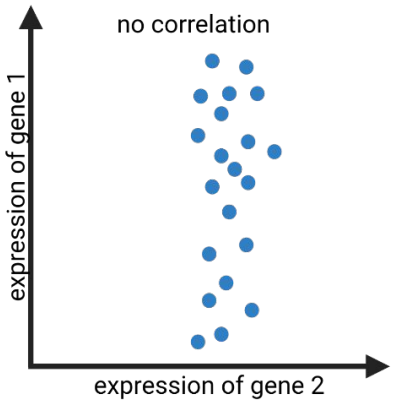
- Correlation does not allow conclusions about the direction i.e. cause and consequence
- Guilt-by-association is frequently used to find genes in a pathway/network
- Correlation can be caused by a direct or indirect connection

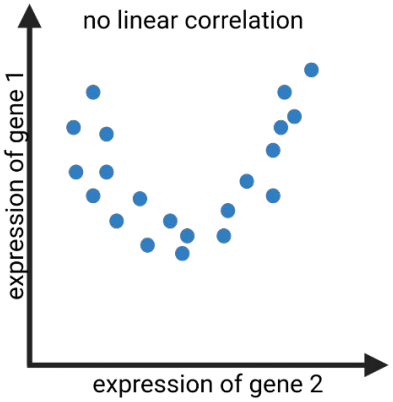
Examples

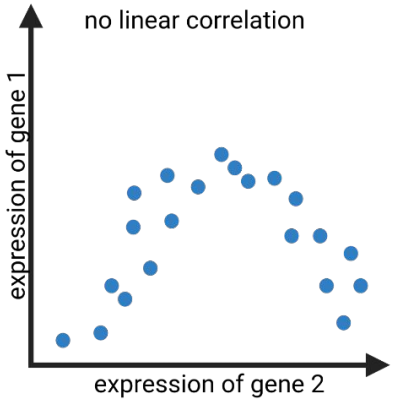

strong positive correlation
expression of gene 1
expression of gene 2

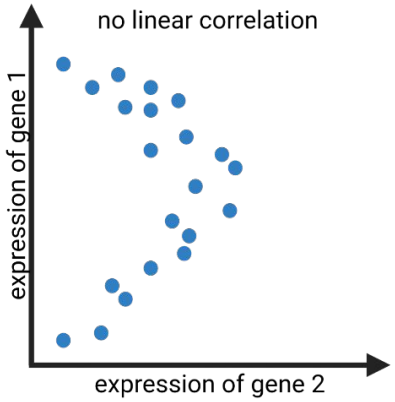

strong negative correlation
expression of gene 1
expression of gene 2

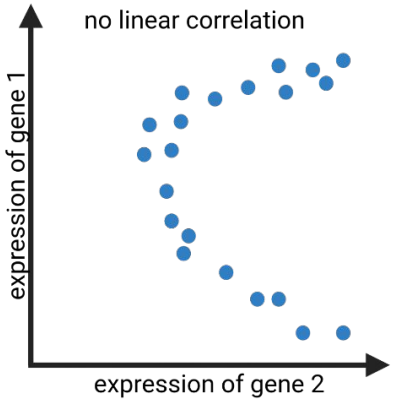

no correlation
expression of gene 1
expression of gene 2


no correlation
expression of gene 1
expression of gene 2


no linear correlation
expression of gene 1
expression of gene 2

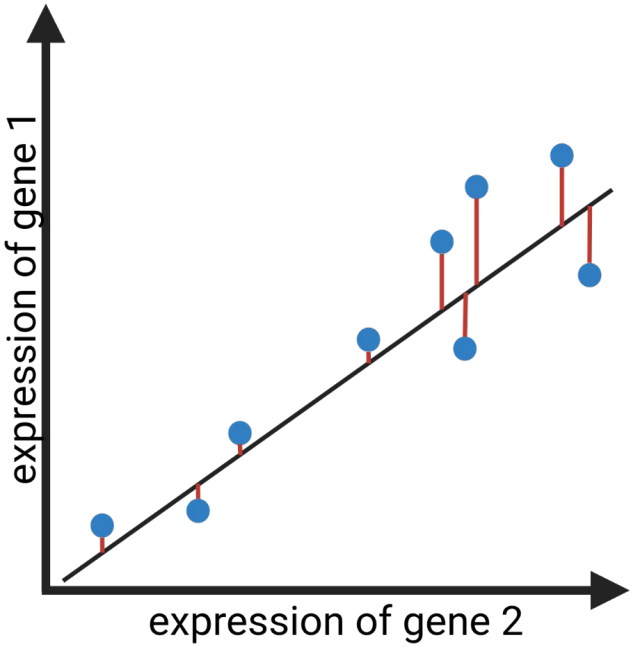

no linear correlation
expression of gene 1
expression of gene 2

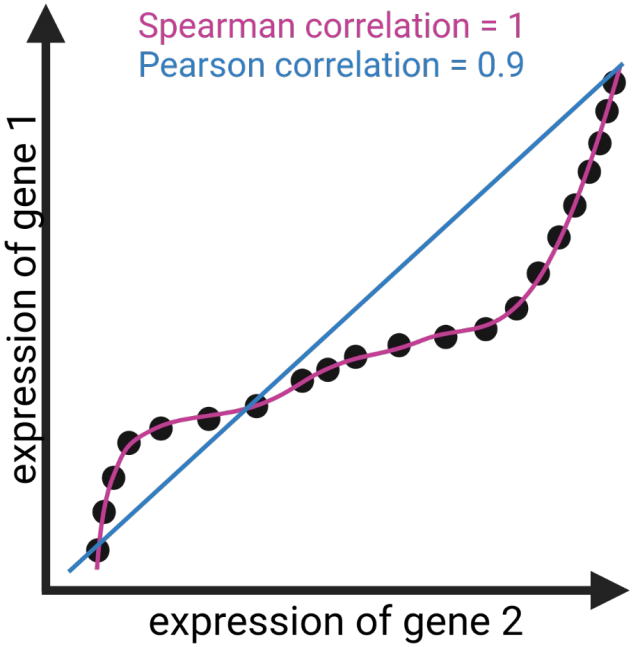

no linear correlation
expression of gene 1
expression of gene 2


no linear correlation
expression of gene 1
expression of gene 2

Types of correlation coefficients

- Pearson is only suitable for linear correlation
- Spearman is more tolerant towards outliers and non-linear correlations





Coexpression network connection

- Calculation of pairwise correlation coefficients of gene expression
- Strength of network edges based on correlation coefficient
- Correlation coefficient is visualized by line width

MYB12

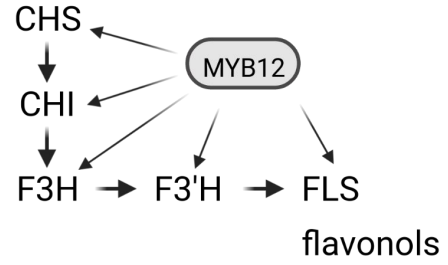
genes	correlation
CHS	0.7
CHI	0.5
F3H	0.45
F3'H	0.6
FLS	0.9

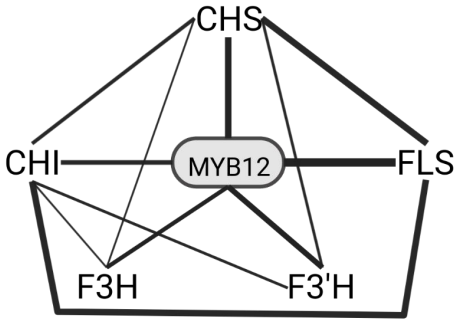
CHS

genes	correlation
CHI	0.4
F3H	0.2
F3'H	0.3
FLS	0.7

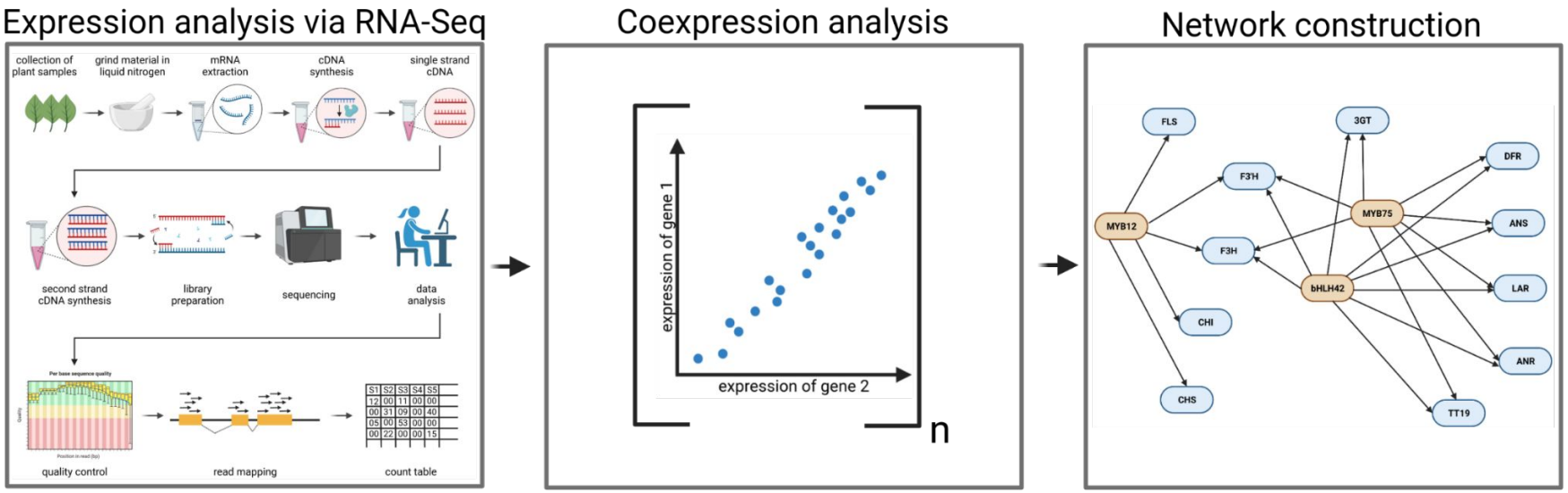
CHI

genes	correlation
F3H	0.2
F3'H	0.3
FLS	0.7



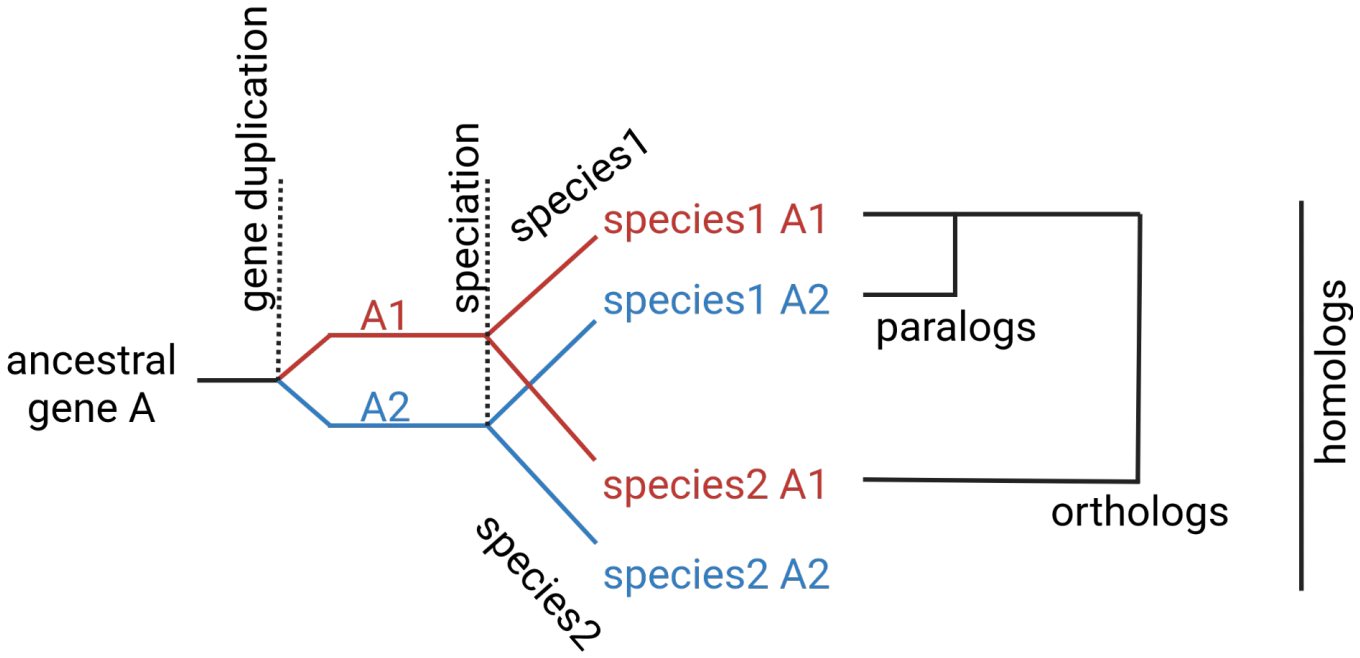


Summary: co-expression workflow



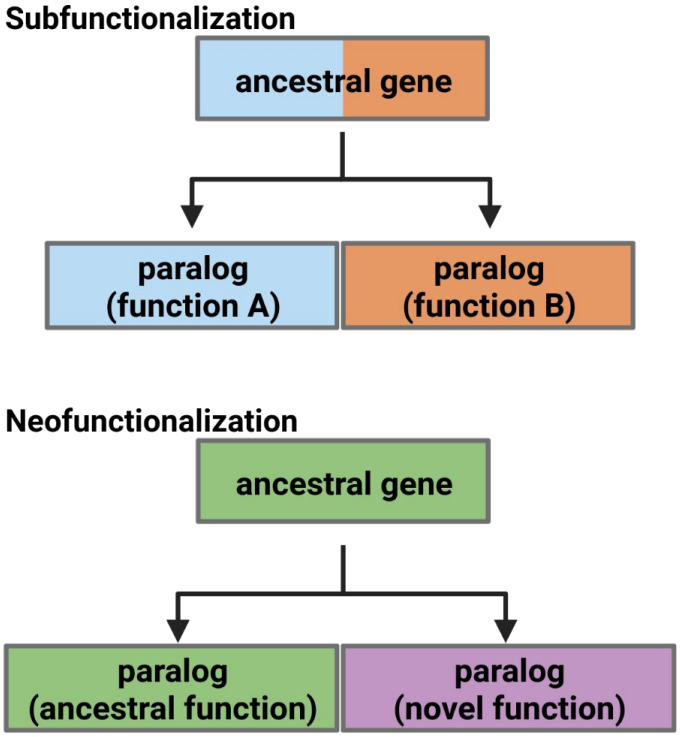
Phylogenetic relationships of genes

- Orthologs are same genes in different species
- Paralogs are gene copies in one species

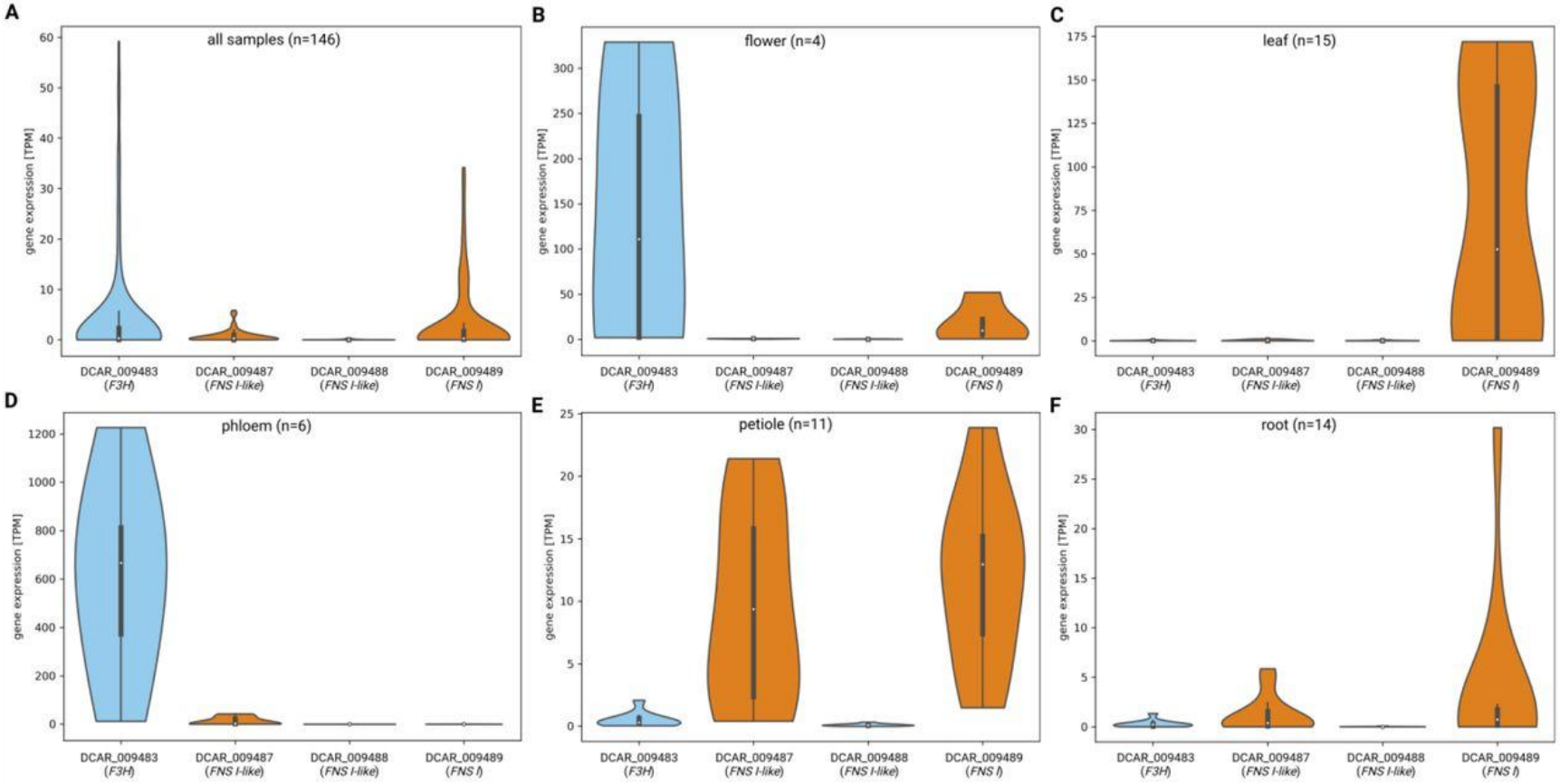


Sub/Neofunctionalization

- Sub/neofunctionalization can be achieved through changes in gene expression following the duplication
- Gene copies can be restricted to specific tissues/conditions

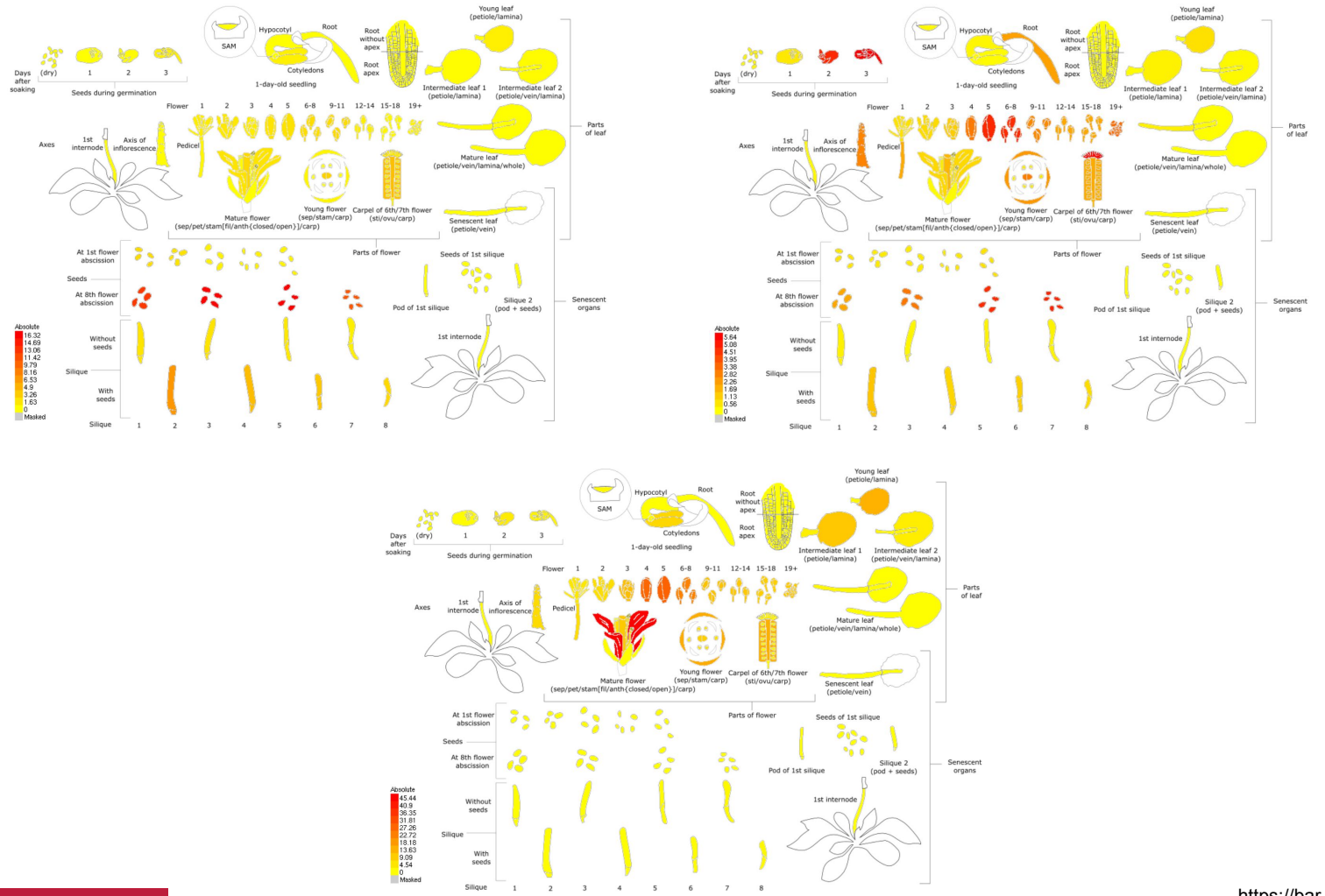


Example: *FNS I* sub/neofunctionalization through expression



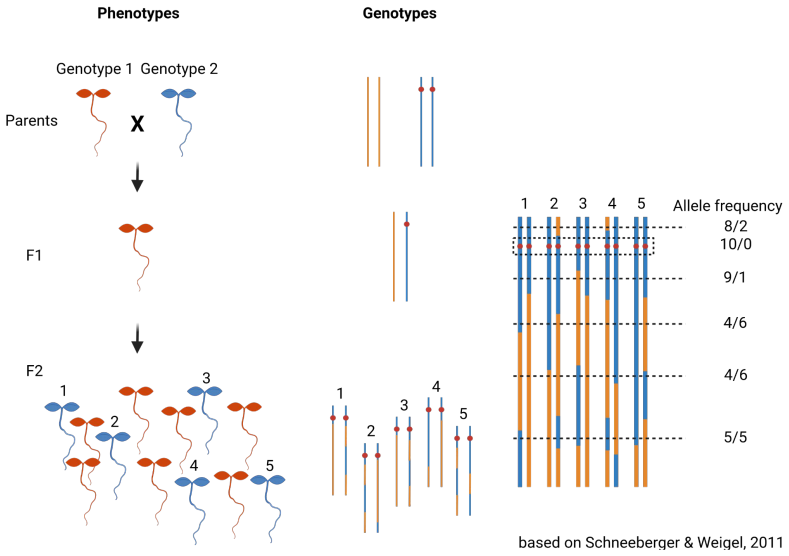
https://doi.org/10.1371/journal.pone.0280155

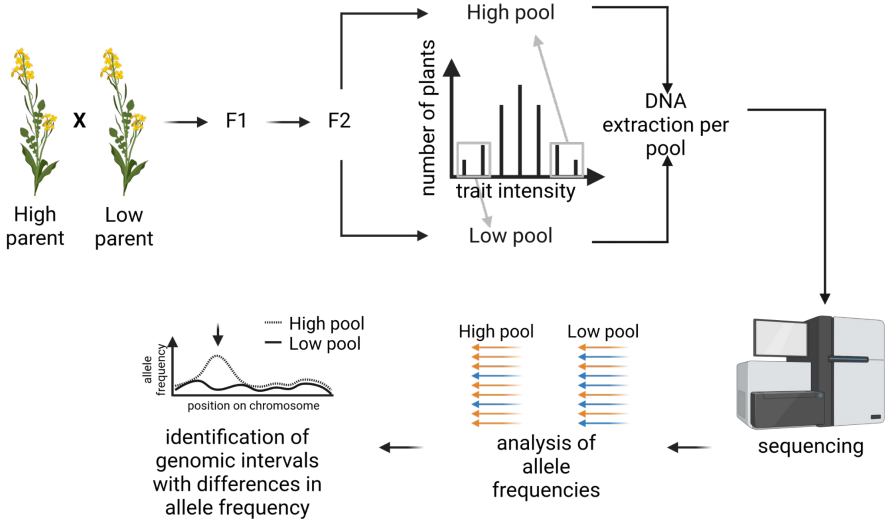
Example: MYB11/12/111



https://bar.utoronto.ca/thalemine

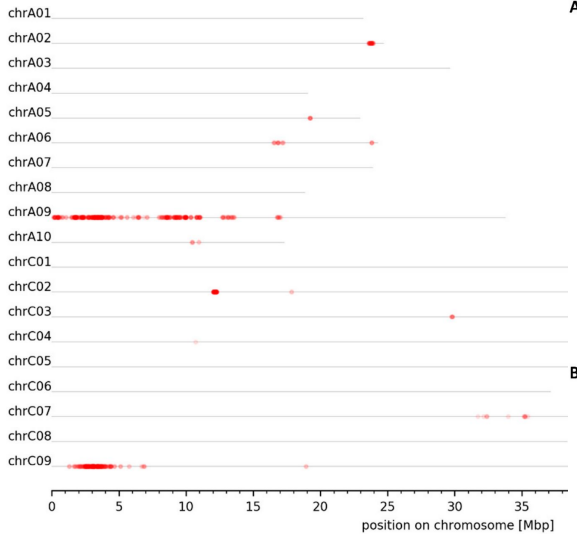
Mapping-by-sequencing

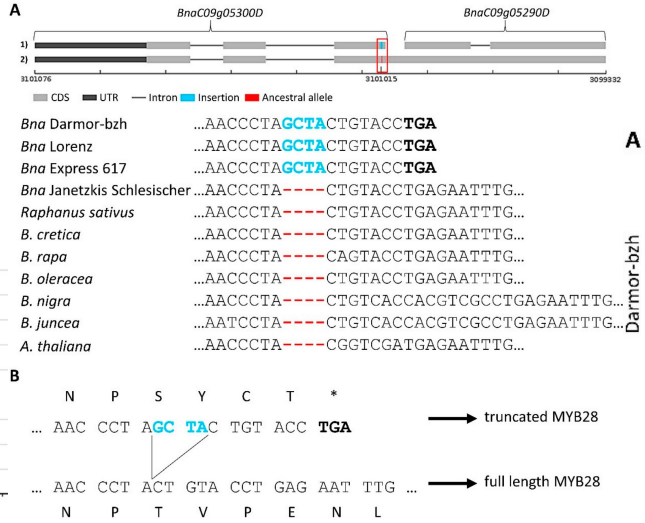


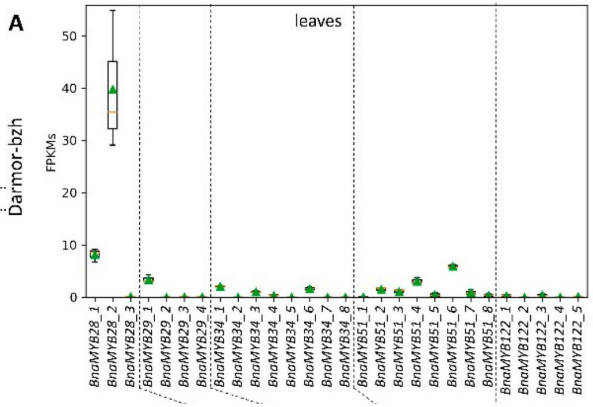


MBS support for candidate gene search

- Mapping-by-sequencing (MBS) reveals genomic region responsible for a trait
- Sequence variant effects can reveal candidate genes
- Identification of causal gene among a few hundred candidates benefits from expression



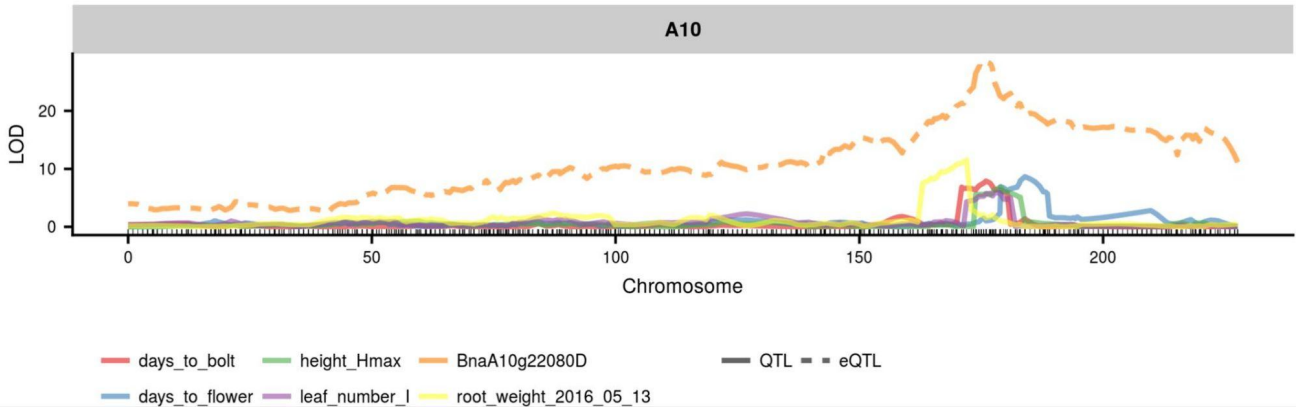




Schilbert & Pucker et al., 2022: https://doi.org/10.3390/genes13071131

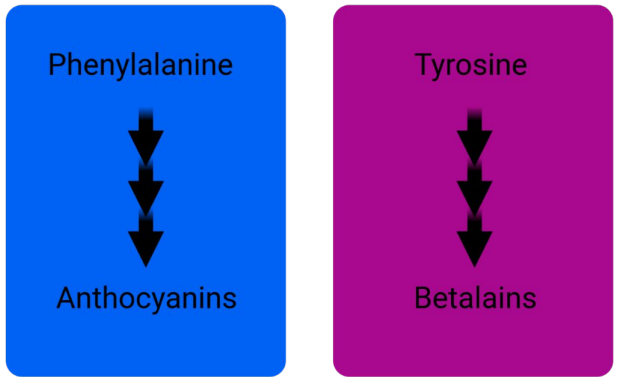
eQTL

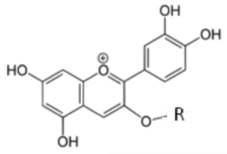
- eQTL = expression quantitative trait locus
- Identification of a genomic region responsible for a trait based on gene expression



https://doi.org/10.3389/fpls.2018.01632

Functional redundancy of anthocyanins and betalains

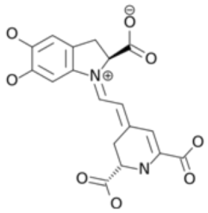




Anthocyanin

Antirrhinum majus

Mirabilis jalapa



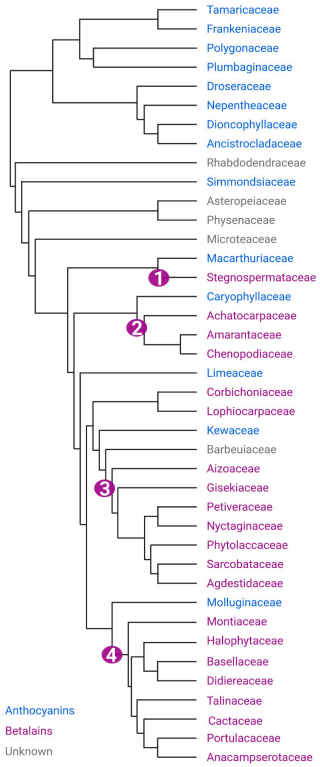
Betalain

Anthocyanin color range

Betalain color range

Brockington *et al.*, 2011

Complex pigment evolution in Caryophyllales



Stegnosperma cubense

Amaranthus cruentus

Mirabilis multiflora

Echinocereus pentalophus

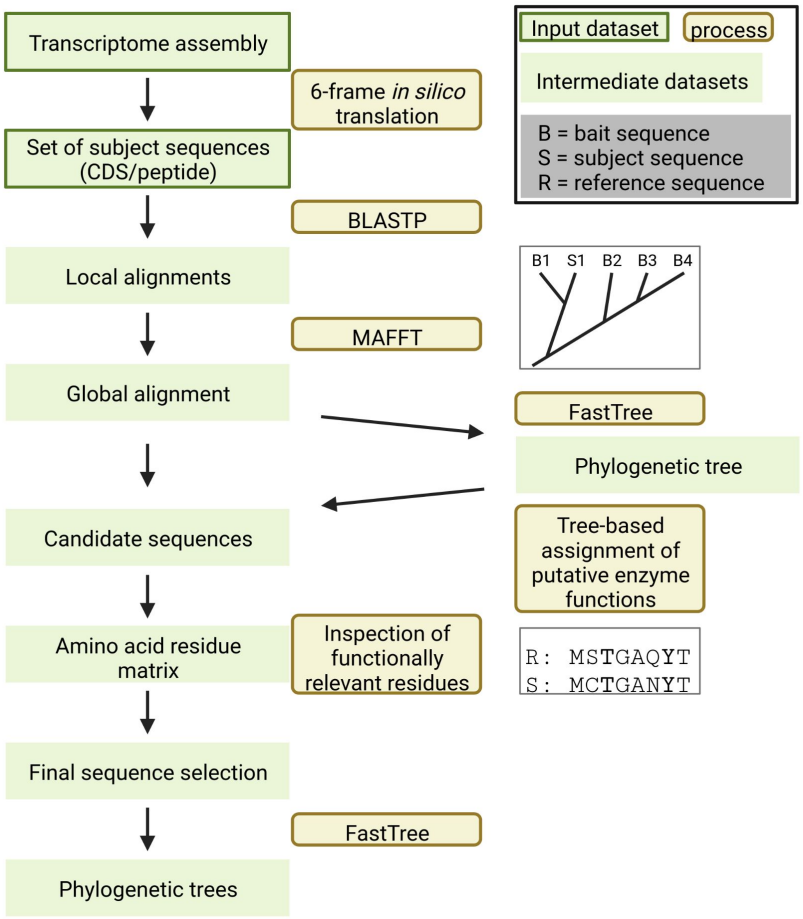
- At least four independent origins of betalain biosynthesis
- Mutual exclusion: anthocyanins and betalains were never observed in same (natural) plants
- Functional redundancy of both pigments

Timoneda *et al.*, 2019
Sheehan *et al.*, 2020
[1] Dick Culbert [2] Stan Shebs [3] Emöke Denes

Hypotheses

- Lack of anthocyanin biosynthesis genes in the Caryophyllales?
- Low/no expression of anthocyanin biosynthesis genes in the Caryophyllales?

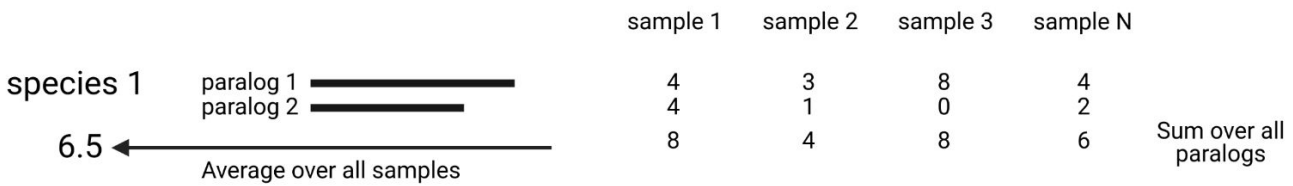
Finding anthocyanin biosynthesis genes

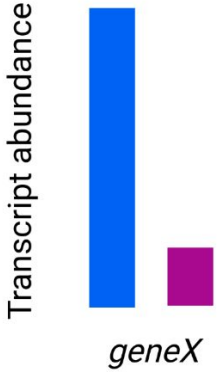


- Identification of sequences across all species in the Caryophyllales
- Knowledge-based Identification of Pathway Enzymes (KIPEs) allows automatic identification of anthocyanin pathways genes
- >300 transcriptome assemblies are covering the families of the Caryophyllales
- Additional genome sequences

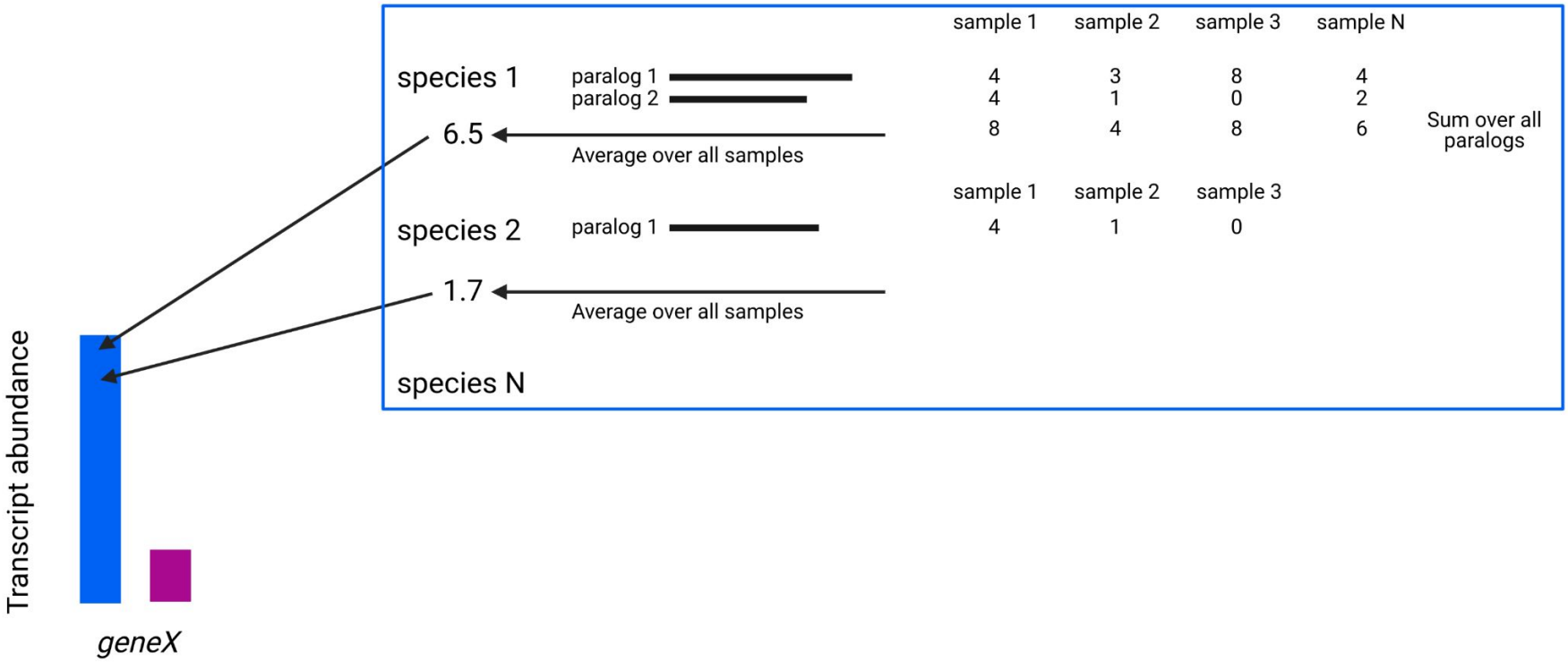
Pucker *et al*., 2020
Rempel & Pucker, 2022

Cross-species transcriptomics

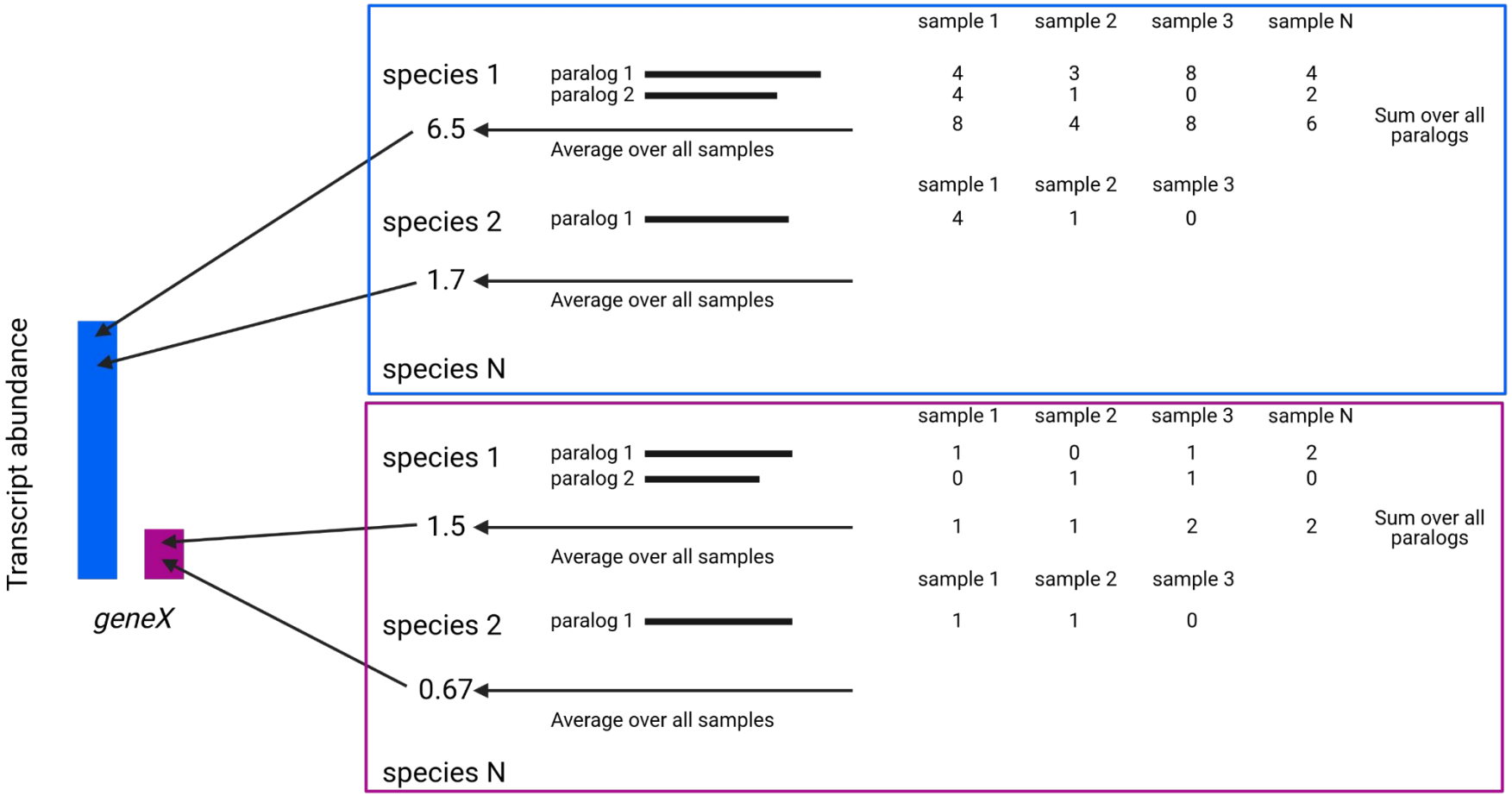




Cross-species transcriptomics

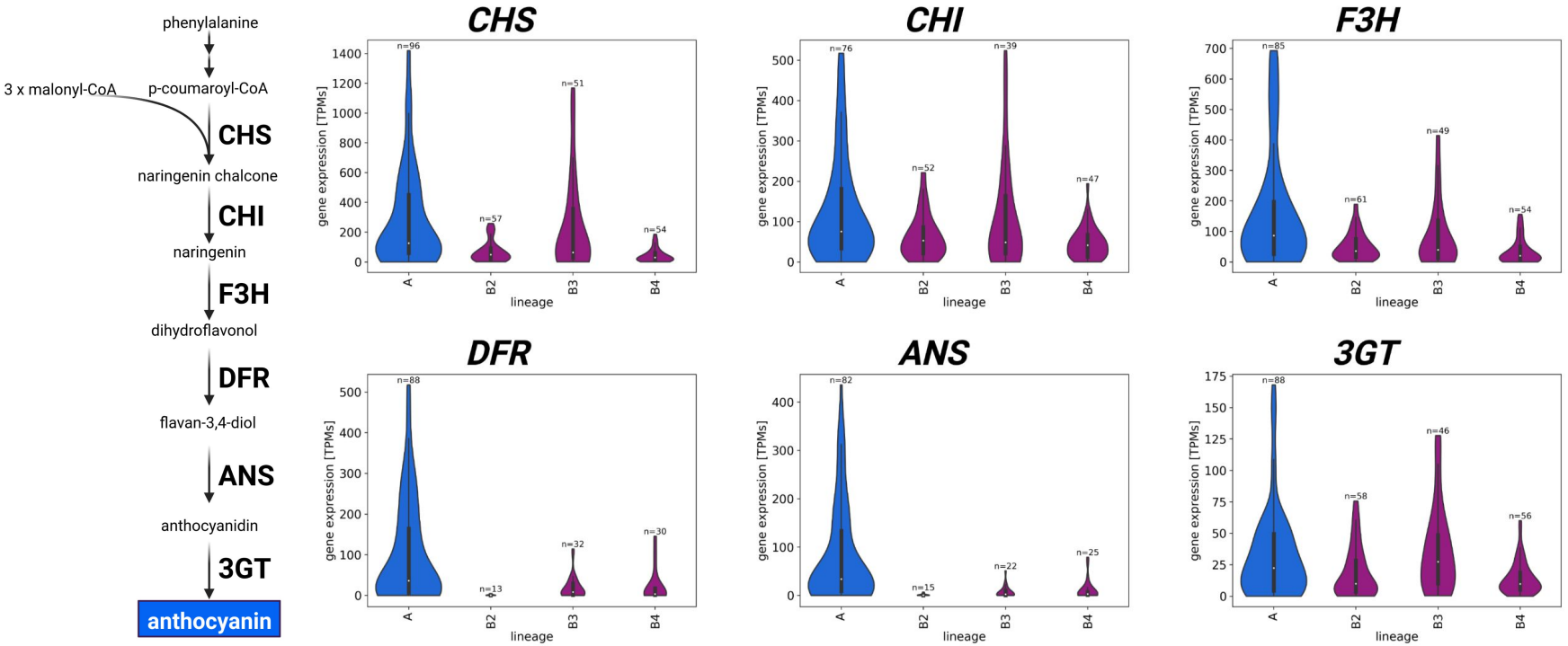


Cross-species transcriptomics



Pucker et al., 2022: 10.1101/2022.10.19.512958

Results of cross-species transcriptomics



Pucker et al., 2022: 10.1101/2022.10.19.512958

Time for questions!

Questions

1. How can RNA-seq support the gene prediction process?
2. Which methods can be applied to study pairwise co-expression?
3. What are the two evolutionary events that can follow gene duplication?
4. Which tool allows an inspection of tissue-specific gene expression?
5. What is an eQTL?