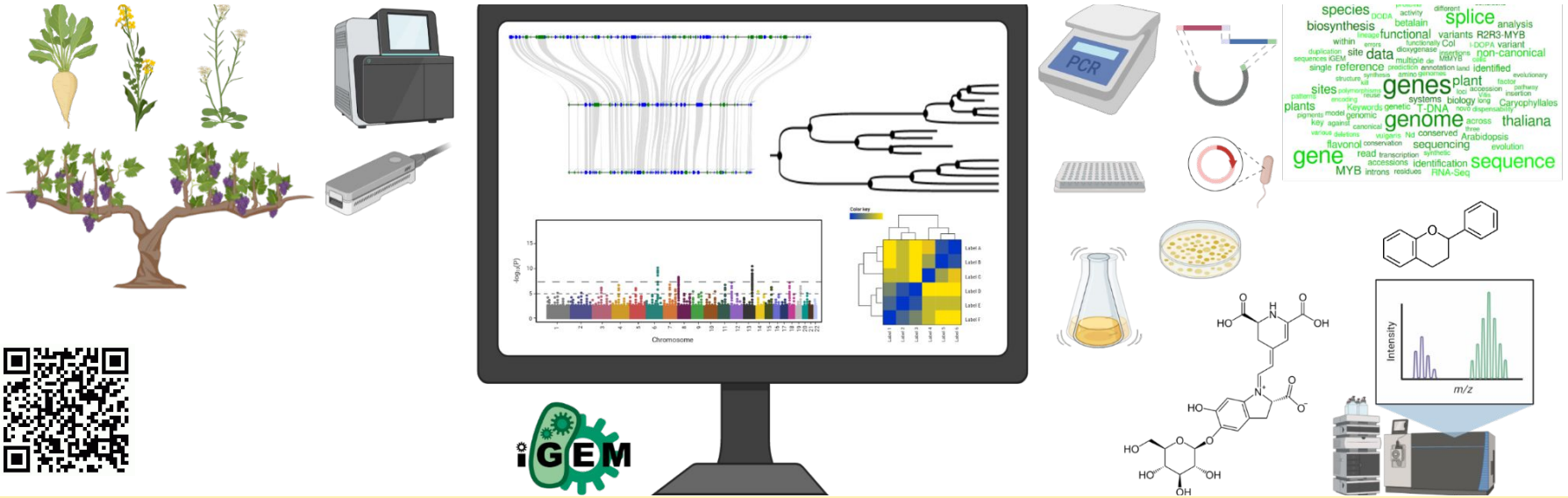
Technische Universität Braunschweig



Assembly and annotation of a genome sequence

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

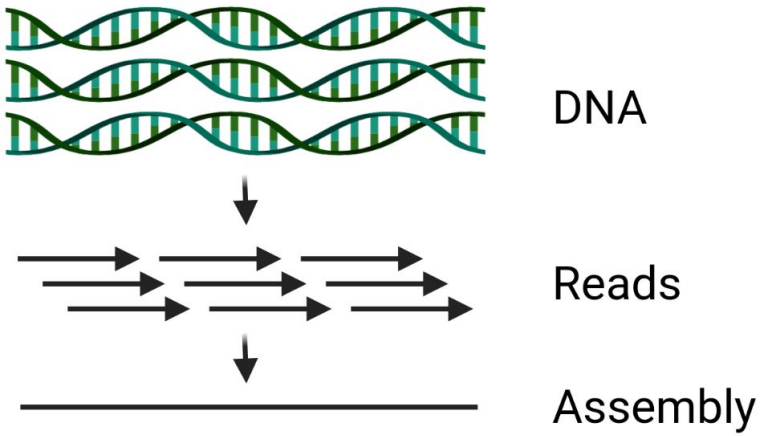
- Questions: Feel free to ask at any time

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

Genome sequence assembly

The assembly problem

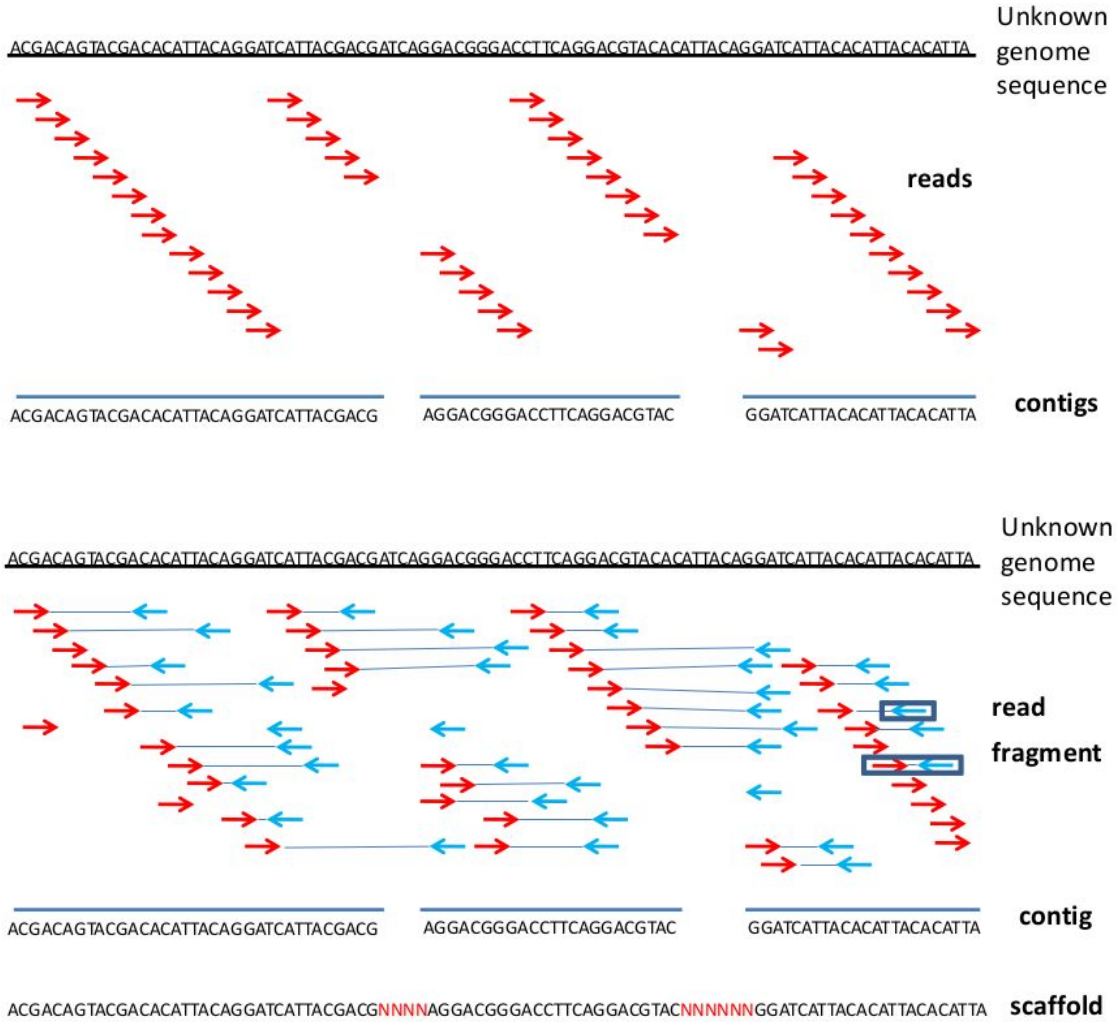
- Reads are shorter than the chromosome - even long reads
- Multiple copies of the genome (DNA exist that can be subjected to sequencing
- Assembly = putting sequence pieces together (finding the common string of all substrings)
- Genome = DNA in a cell
- Genome sequence = representation of the DNA in a cell; stored in FASTA files




```
>TRINITY_DN100016_c0_g1_i1
MPKKSSNIQQNNQNRIKRKGRPPKKKKYVQQIDSSDEDILSVRHASTRPRIISIRRNEIPMRPEIHI
>TRINITY_DN100019_c0_g1_i1
MPPKAADKKPAAKAPATASKAPEKKDAGKKTAATGDKKKRTKARKETYSSYIYKVLKQVHPDTGISN
>TRINITY_DN10001_c0_g1_i2
MAKVGNPIVDIETDGSVNEPESSEKNIEVSSSSTQAPESTNTTELLVNEKKAFSLATPAVRRVAREH
>TRINITY_DN100025_c0_g1_i1
MVENQDGCFFKPGWKEFVRSNDLEGGDFLVFNLVDKISYQVVIFDGTCCPKDLCPFSIMNPIFQHLR
>TRINITY_DN10002_c0_g1_i1
MSDELNQVFQRCREQKRPVFVAFVTAGYPDSEETVDILLGLEAGGADIIELGIPFTDPMVDGKTIQD
>TRINITY_DN100061_c0_g1_i1
MQQVVAKLKAIITKTNVTNENSPVENSSSSTSATSSINNSLHGDLSRVFDNMELESNVSNSSISSNI
```

What is a contig/scaffold?

Contigs, scaffolds, pseudochromosomes



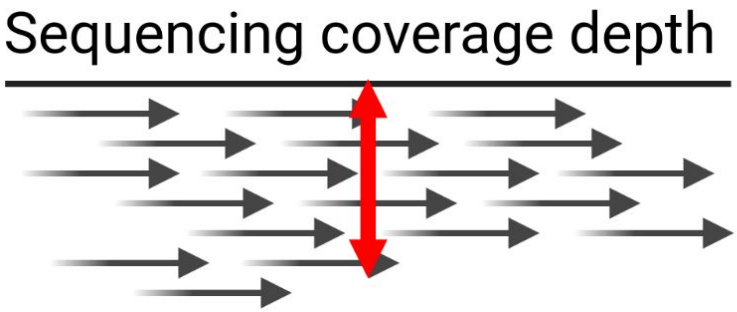
Contig = continuous sequence

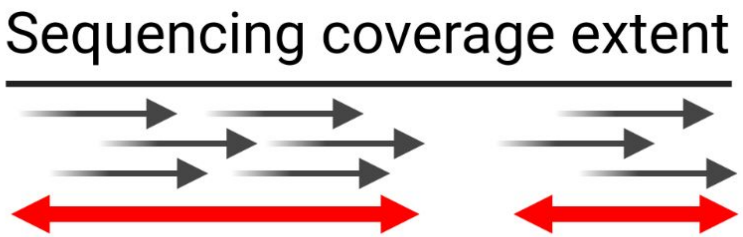
Scaffold = compilation of contigs with interleaved, unknown sequence (gaps)

Gaps = regions between contigs that are represented by Ns

Pseudochromosomes = scaffolds representing an entire chromosome

Sequencing coverage depth vs. coverage extent





Sequencing coverage depth

- Coverage depth = average number of times a given base is being sequenced

- Calculation:
  - N = number of reads
  - L = read length in base pairs
  - G = genome size in base pairs
  - Coverage depth d = N X L / G

- Coverage (depth) reflects total amount of sequencing data

- Coverage (depth) is very important parameter for sequencing projects

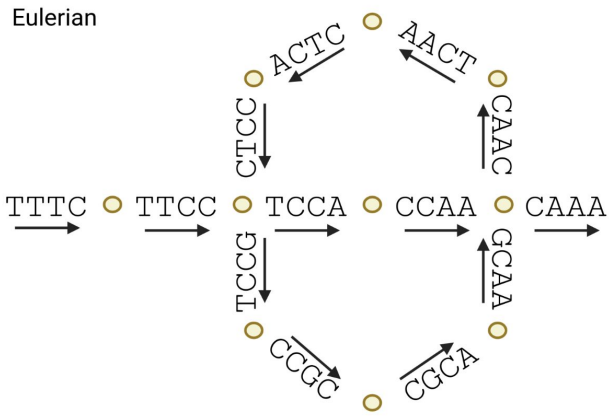
Sequencing coverage extent

- Coverage extent = ratio of genome covered by at least one base
- Informative to calculate required sequencing depth for a project
- Coverage extent follows a Poisson distribution
- Calculation of coverage extent (c):
  - Non-coverage extent: $P(X=0) = e^{-c}$
  - Probability of coverage extent: $P(X>0) = 1 - e^{-c}$
- Complete coverage of genome requires $G \times e^{-c} < 1$
- Larger genomes required higher sequencing depth
- Real coverage extent is often suffering from sequencing bias

De Brujin graph (DBG)

- 1) Reads are broken into smaller k-mers
- 2) K-mers represented in a “De Bruijn graph”
- 3) Inference of genome sequence from graph
- Paradigm used in many assemblers: Velvet, ABySS, AllPath-LG, SOAPdenovo
- Complexity:
  - Number of nodes and links equal to genome size
  - Eulerian path problem is ‘easy’ to solve

```
TTTCCGCAACTCCAA
TTTC
 TTCC
  TCCG
   CCGC
    CGCA
     GCAA
      CAAC
       AACT
        ACTC
         CTCC
          TCCA
           CCAA
```

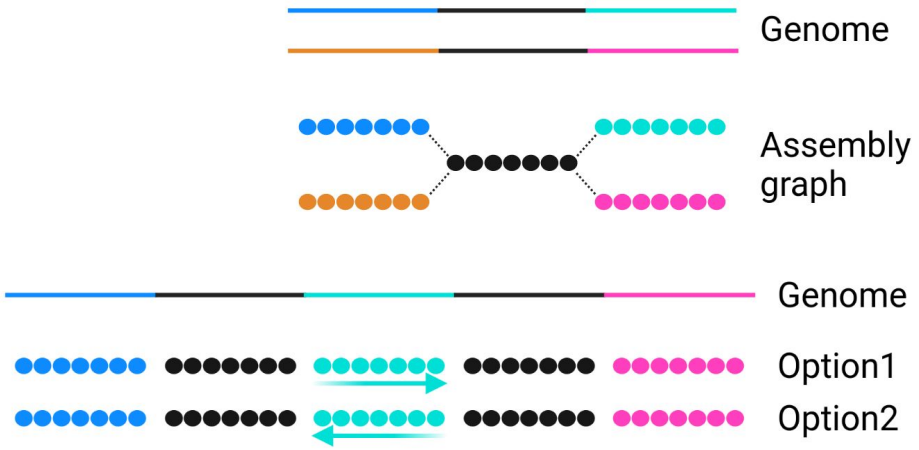


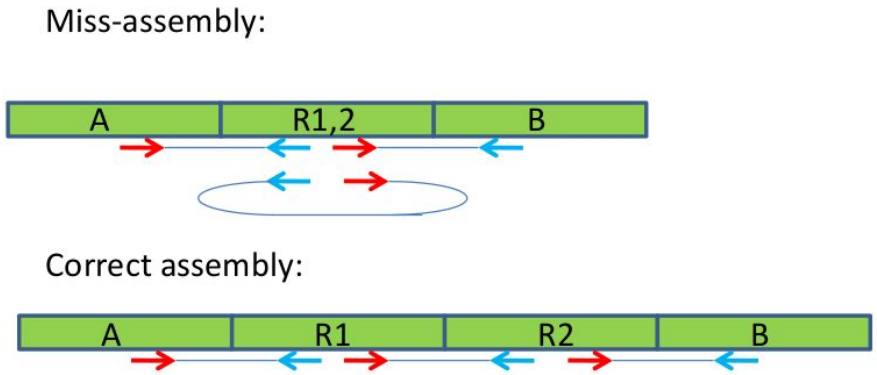
K-mer size

- Larger k-mers increase the assembly continuity by spanning repeats
- Larger k-mers are more sensitive to sequencing errors
- Removal of low abundance k-mers (caused by sequencing errors)
- K-mer size should be 0.5 to 0.75 of the read length
- Typical k-mer sizes:
  - 87 or 97 for 2x150bp reads

Assembly challenges

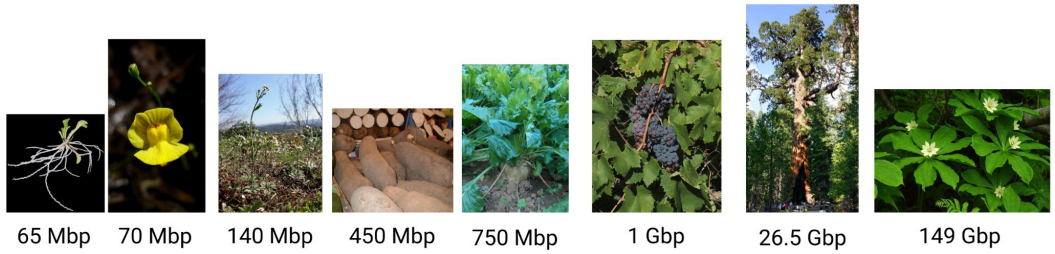
- Collapsed repeats
- Inversions
- Overstretched repeats





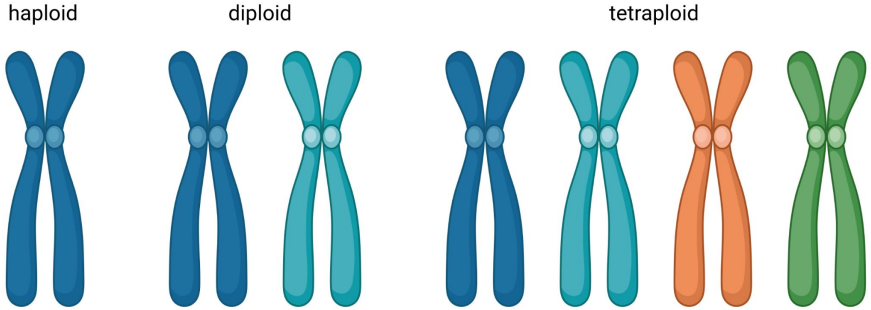
Assembly challenges (2)

- Genome size: variation from 65 Mbp to 149 Gbp



Genlisea violacea: https://commons.wikimedia.org/wiki/File:Genlisea_violacea_giant.jpg
Utricularia gibba: https://commons.wikimedia.org/wiki/File:Utricularia_gibba_flower_01.jpg
Arabidopsis thaliana: https://commons.wikimedia.org/wiki/File:Arabidopsis_thaliana_sl10.jpg
Dioscorea dumetorum: https://commons.wikimedia.org/wiki/File:YamsatBrixtonMarket.jpg
Beta vulgaris: https://de.wikipedia.org/wiki/Zuckerr%C3%BCbe#/media/Datei:Zuckerr%C3%BCbe.jpg
Vitis vinifera: https://commons.wikimedia.org/wiki/File:Vitis_vinifera_20120823.jpg
Sequoia giganteum: https://commons.wikimedia.org/wiki/File:Grizzly_Giant_Mariposa_Grove.jpg
Paris japonica: https://commons.wikimedia.org/wiki/File:Paris_japonica_Kinugasasou_in_Hakusan_2003_7_27.jpg

- Ploidy: haploid/diploid genomes are much easier to analyze than polyploid genomes



Kress et al., 2022: 10.1073/pnas.2115640118

Assembly polishing

ONT assembly ...ACGTTACGGTACGACATGACGTAAAAAAAAACGATAGTACGTGTTAGCGTACGTACGTAACGTT...

Illumina reads

```
          GACGTAAAAAAAA-CGATAGTACGTGTTAGCGTACGTAC
       ATGACGTAAAAAAAA-CGATAGTACGTGTT
            CGTAAAAAAAA-CGATAGTACGTGTTAGCGTAC
    ACATGACGTAAAAAAAA-CGATAGTACGT
          CGTAAAAAAAA-CGATAGTACGTGTTAGCGT
       GACGTAAAAAAAA-CGATAGTA
```

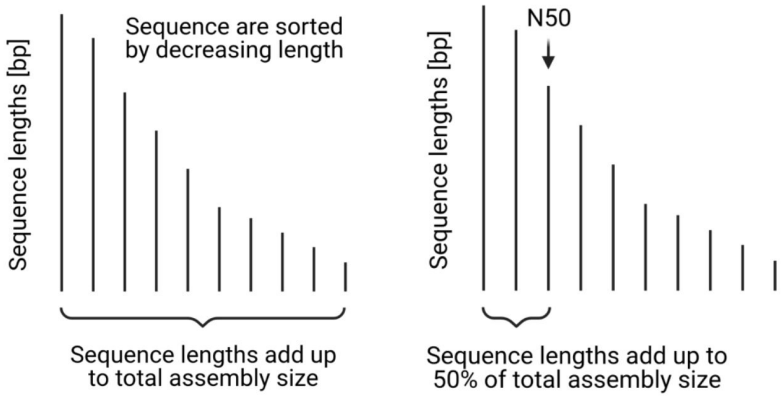
Assembly evaluation

- Continuity: Does the assembly represent a genome in a small number of contigs?
- Completeness: Are all parts of the genome represented?
- Correctness: Is the assembly a correct representation of the genome?

Evaluation: continuity

- Check assembly continuity: calculation based on sequences in FASTA file
- Number of contigs, assembly size, N50

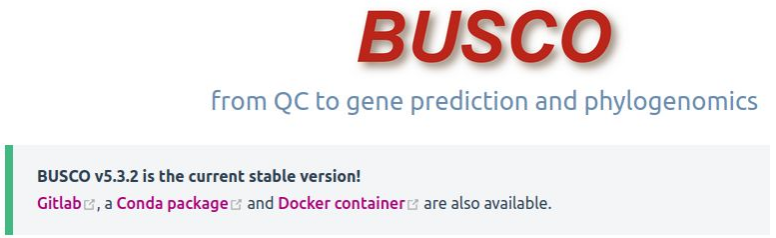
assembler	Canu	FALCON	Miniasm	Flye
number of contigs	69	26	72	44
assembly size	123.5 Mbp	119.5 Mbp	120.2 Mbp	117 Mbp
maximal contig length	15.9 Mbp	15.9 Mbp	14.3 Mbp	14.9 Mbp
N50	13.4 Mbp	9.3 Mbp	8.6 Mbp	10.6 Mbp
N90	2.9 Mbp	2.8 Mbp	1.4 Mbp	2.5 Mbp



Pucker et al., 2019: 10.1371/journal.pone.0216233

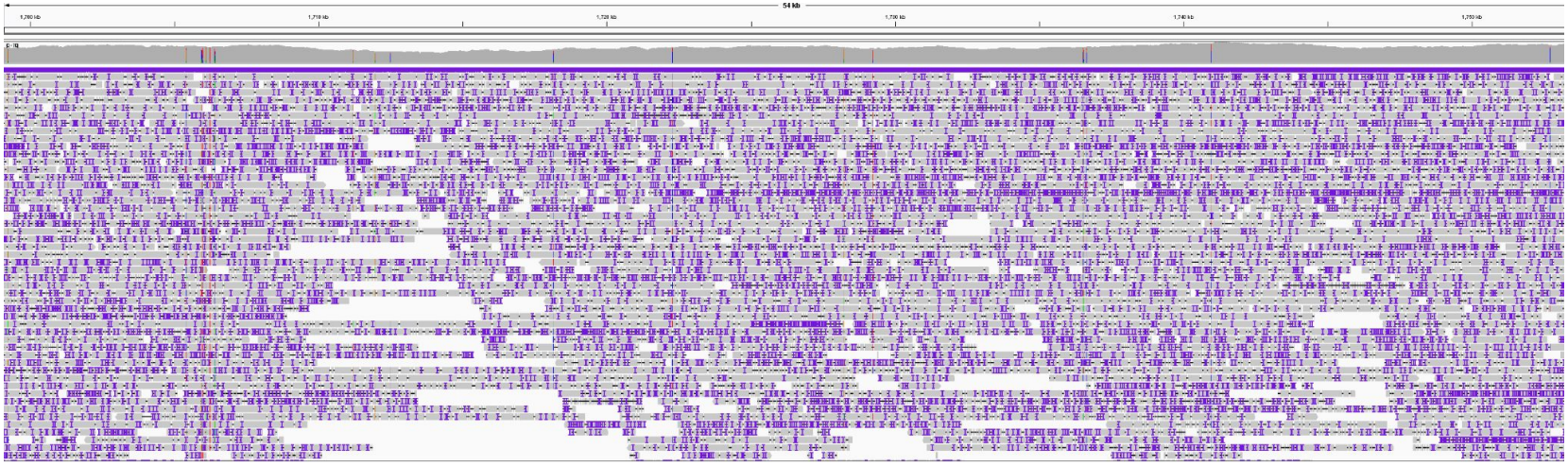
Evaluation: completeness

- Check assembly completeness: inspection for presence of conserved genes
- BUSCO = Benchmarking Universal Single-Copy Orthologue
- BUSCO genes are used to assess assembly completeness



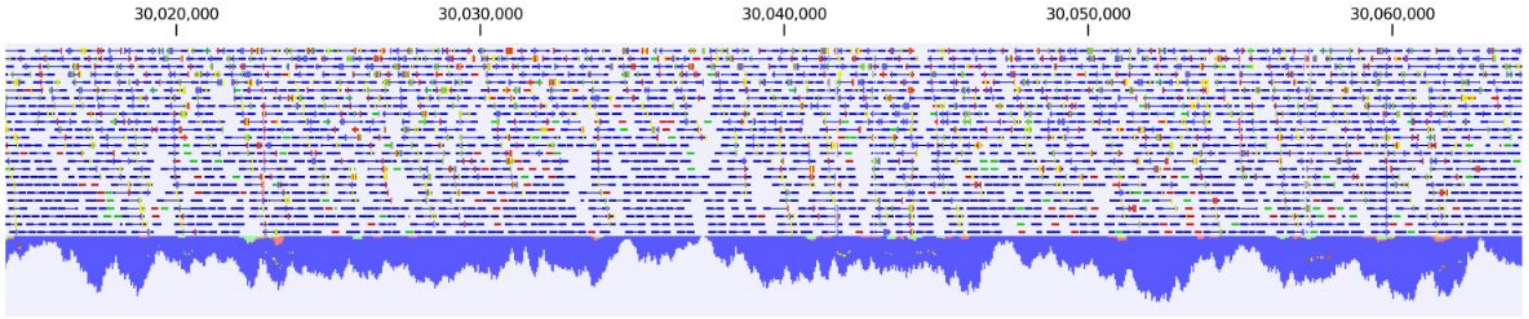
Evaluation: assembly correctness

- Check assembly correctness: analyses of read mappings
- Integrated Genomics Viewer (IGV) can visualize read mappings
- Tools: REAPR, SQUAT



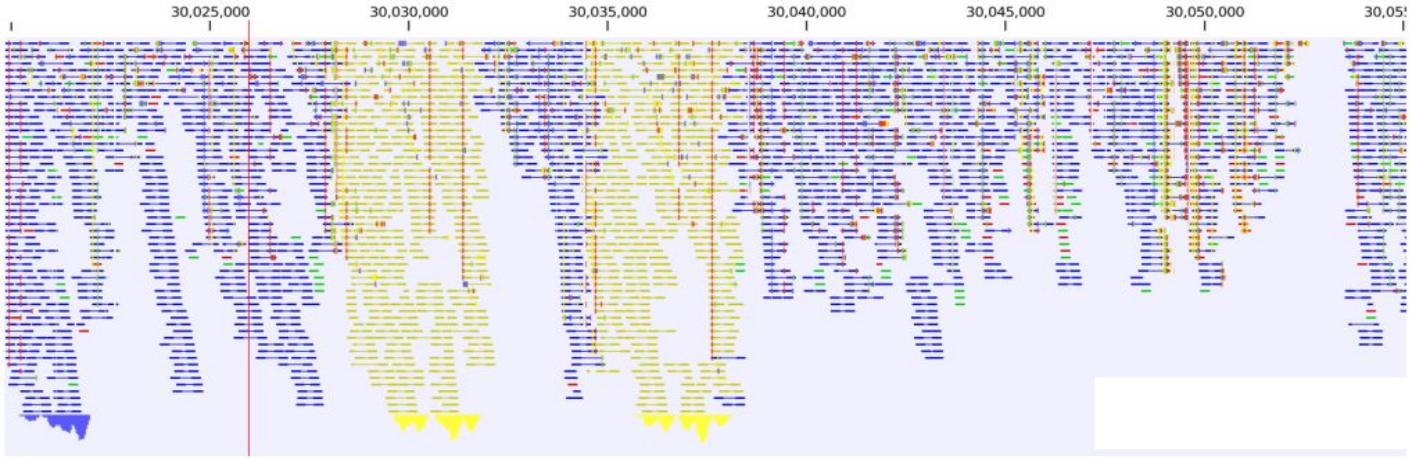
IGV: Thorvaldsdottir et al., 2013: 10.1093/bib/bbs017
REAPR: Hunt et al., 2013: 10.1186/gb-2013-14-5-r47
SQUAT: Yang et al., 2019: 10.1186/s12864-019-5445-3

Read mappings (1)

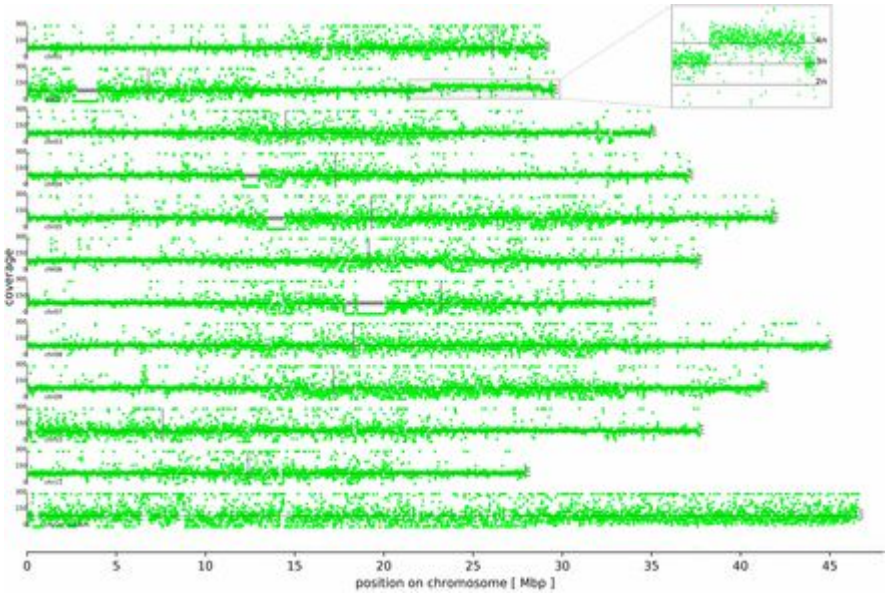


Read mapping of paired-end sequenced fragments (blue) to assembly

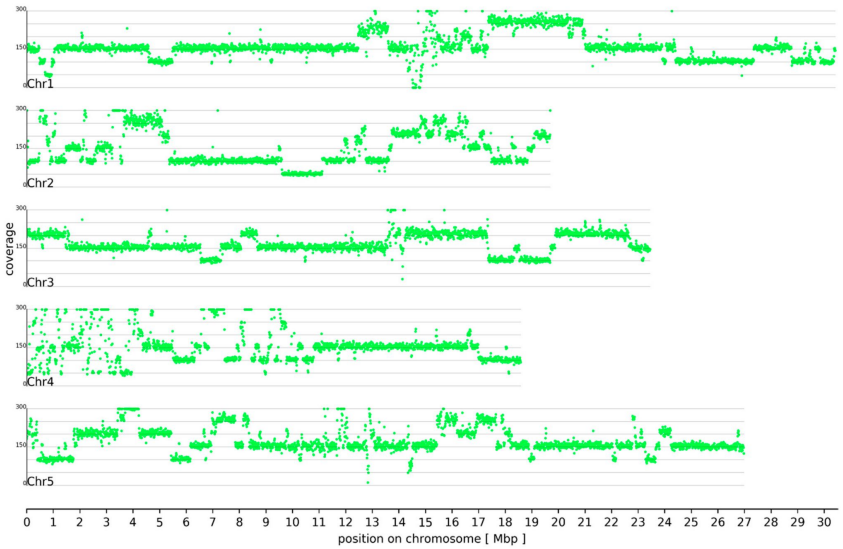
Coverage is too high to show all individual fragments at some positions



Read mappings (2)



Musa acuminata (banana) read mapping



Arabidopsis thaliana At7 read mapping

Pucker et al., 2019: 10.3390/genes10090671
Busche & Pucker et al., 2020: 10.1534/g3.119.400847

Advantages of long reads

- Span larger regions and enable assembly of repeats
- Specific mapping to repetitive regions possible
- Generation of larger contigs (no scaffolding)
- Contigs can represent entire chromosomes

Long read assemblers

- Canu: https://github.com/marbl/canu
  Nurk et al., 2020: 10.1101/gr.263566.120

- Miniasm: https://github.com/lh3/miniasm
  Li, 2016: 10.1093/bioinformatics/btw152

- FALCON: https://github.com/PacificBiosciences/FALCON
  Chin et al., 2016: 10.1038/nmeth.4035

- Shasta: https://github.com/chanzuckerberg/shasta
  Shafin et al., 2020: 10.1038/s41587-020-0503-6

Importance of error rate

- Correction step is computationally extremely intense
  - Computation of all-vs-all alignments

- Direct assembly is possible with >99% raw read accuracy

- Higher accuracy allows to filter read overlaps more strictly

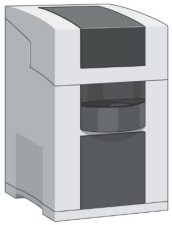
Integration of genetic linkage information (1)

- Classical genetic markers: SSR, CAPS, KASP
  - SSR = Simple Sequence Repeats
  - CAPS = Cleaved Amplified Polymorphic Sequences
  - KASP = Kompetitive Allele Specific PCR

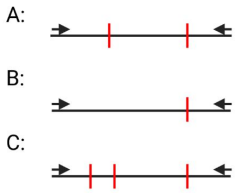
SSR: Simple Sequence Repeats

```
A: CATAGAGAGAGAGAGATGAC
B: CATAGAGAGAGAGATGAC
C: CATAGAGAGATGAC
D: CATAGAGAGAGAGAGATGAC
E: CATAGAGAGAGAGATGAC
F: CATAGAGAGAGAGAGATGAC
G: ACATAGAGATGAC
```

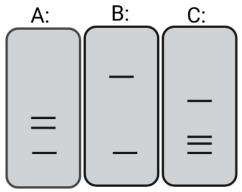
Length analysis via capillary electrophoresis



CAPS: Cleaved Amplified Polymorphic Sequences



PCR & restriction digest



KASP: Kompetetive Allele Specific PCR

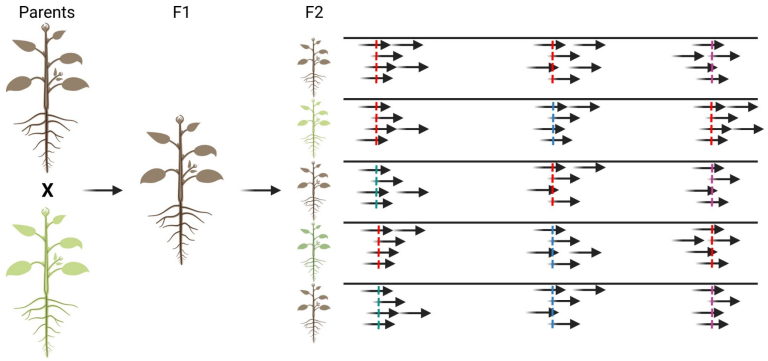
```
CATAGACACTG[G/A]GTTAGAGATGAC
```

qPCR with fluorescently labeled primers

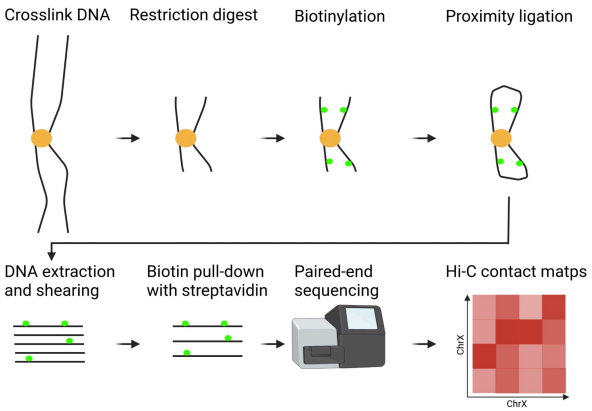
SSR: Holtgräwe et al., 2020: 10.3389/fpls.2020.00156
CAPS: Konieczny & Ausubel, 1993: 10.1046/j.1365-313x.1993.04020403.x
KASP: He et al., 2014: 10.1007/978-1-4939-0446-4_7

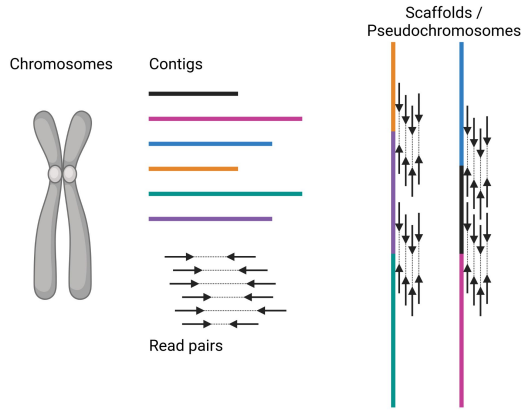
Integration of genetic linkage information (2)

- Genotyping-by-sequencing: SNPs inferred from sequencing data



- Hi-C: chromatin interaction information for long range scaffolding



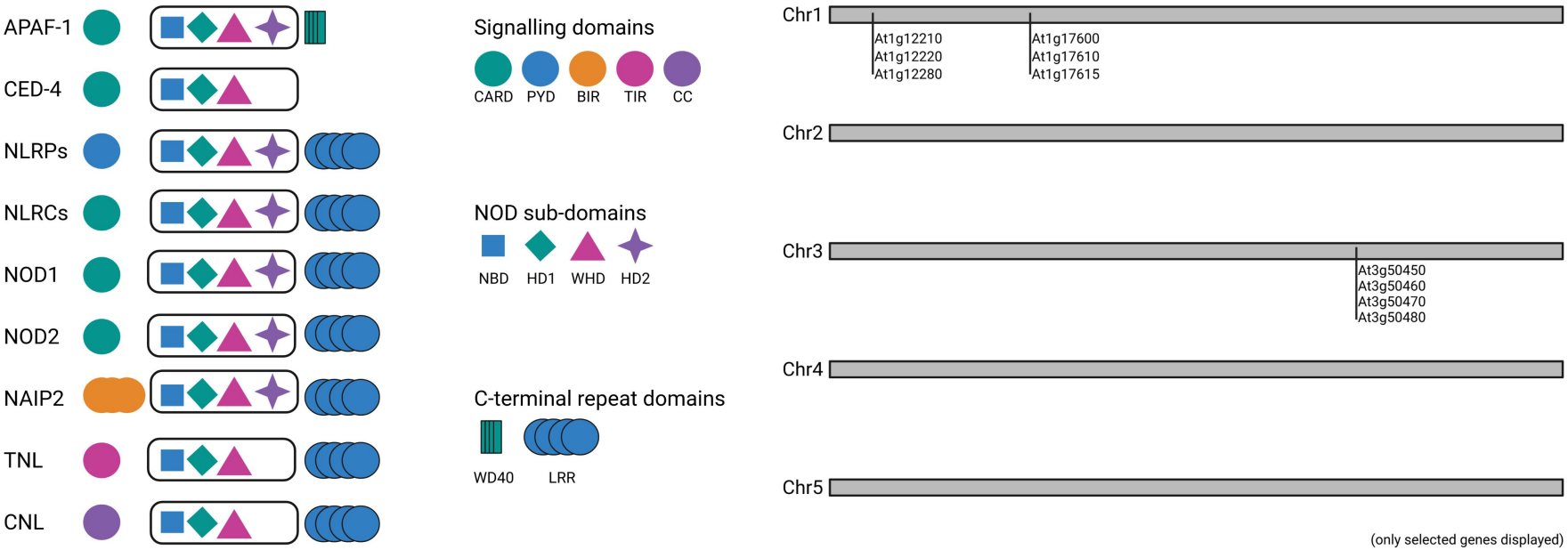


Checking challenging regions

- Resolving the centromeres and NORs are the last big challenges
- Centromeres = repeats in the middle of chromosomes
- Nucleolus Organizing Regions (NORs) = ribosomal RNA encoding repeats (rDNA)
- Checking presence of telomeres at contig ends

NLRome

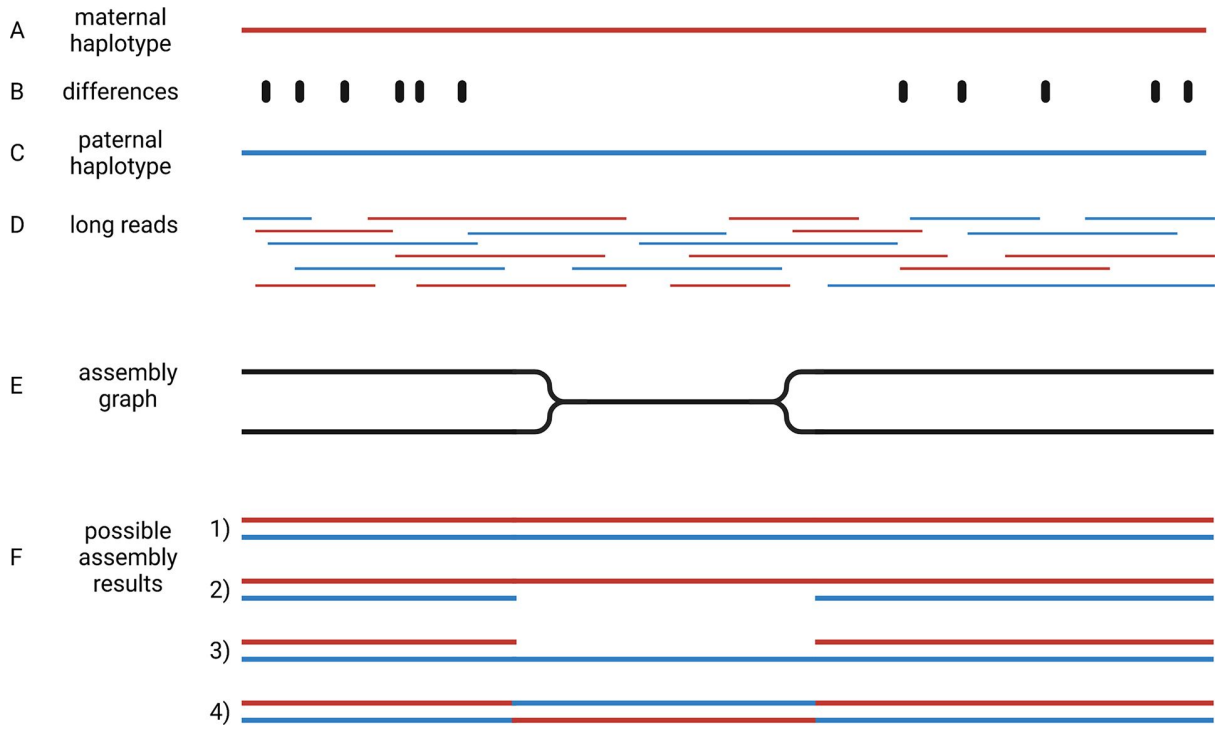
- NLR genes (NLRome) are often clustered in tandem repeat arrays
- NLR gene clusters are particularly tricky to assemble
- NLRome used for benchmarking high quality assembly



Van de Weyer et al., 2019: 10.1016/j.cell.2019.07.038

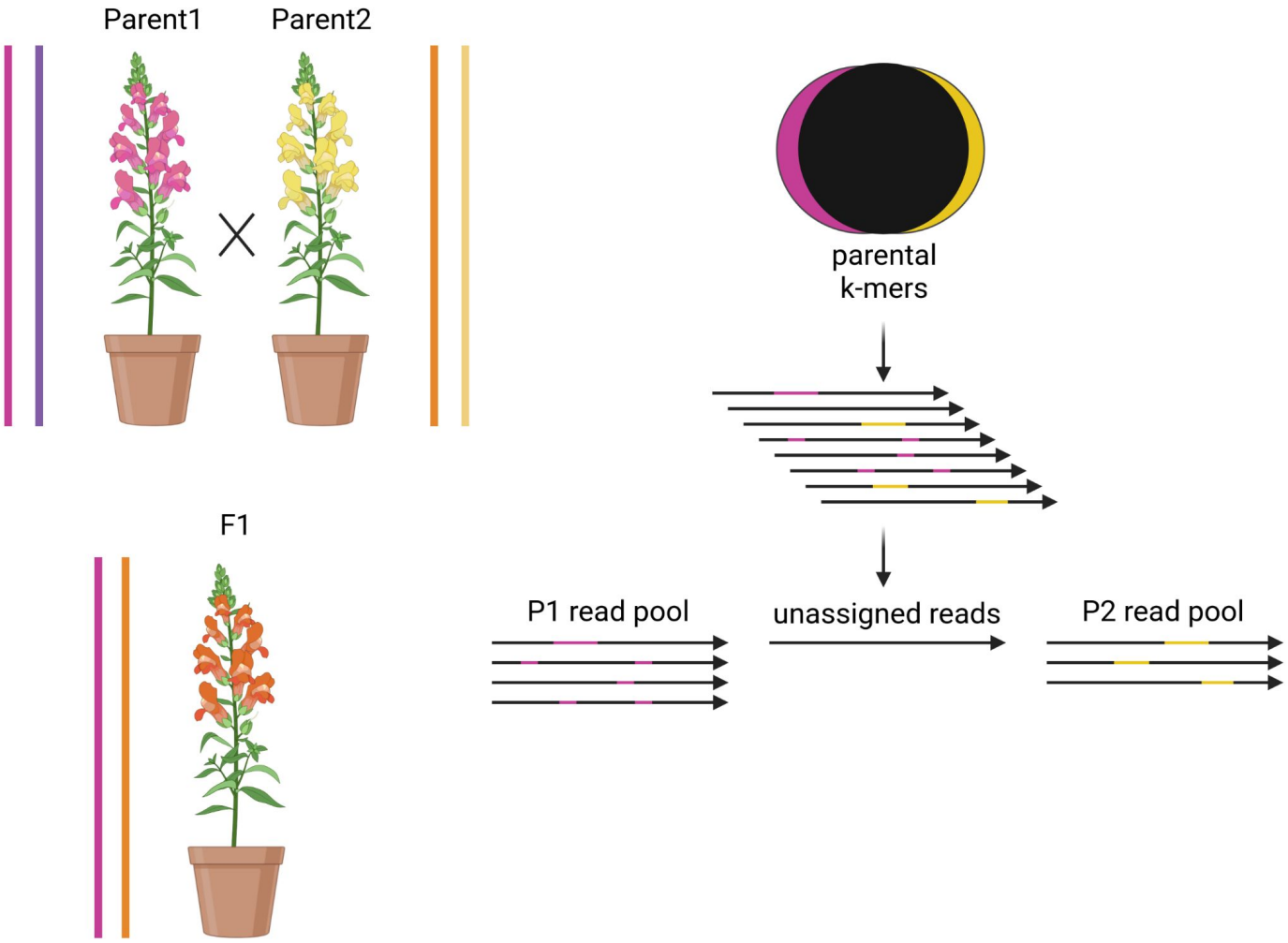
Haplophases

- Haplotype = combination of alleles
- Haplophase = representation of a haplotype



Pucker et al., 2022: 10.1017/qpb.2021.18

TrioBinning



Koren et al., 2018: 10.1038/nbt.4277

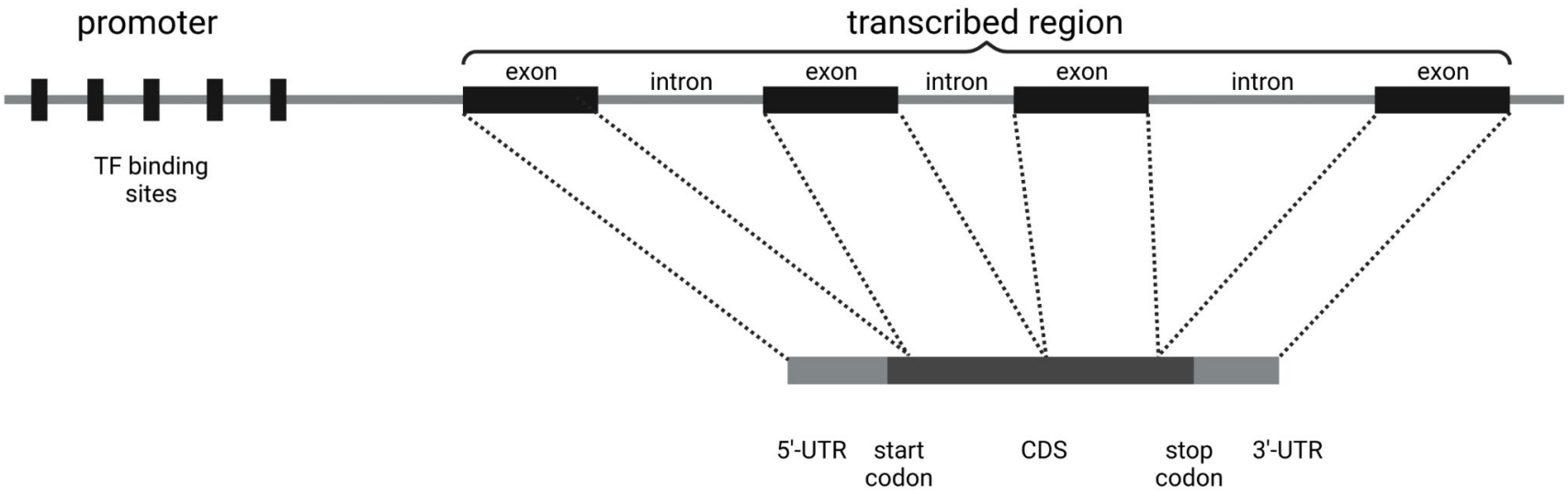
Genome sequence annotation

Finding genes in a genome sequence

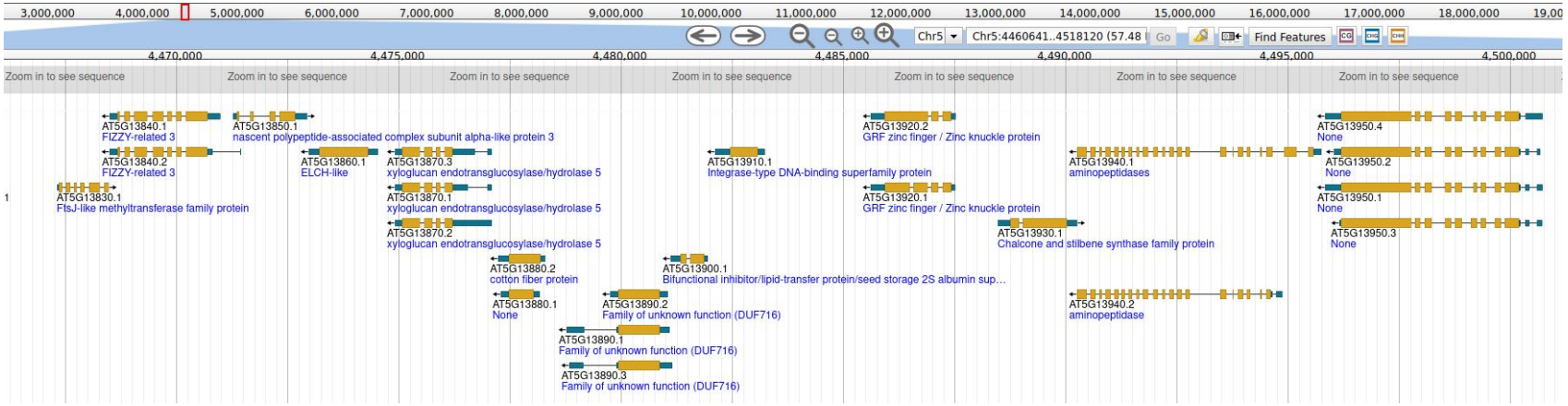
Siadjeu & Pucker et al., 2020: 10.3390/genes11030274

Plant gene structure

CDS = (Protein) Coding Sequence
ORF = Open Reading Frame
UTR = UnTranslated Region
TF = Transcription Factor

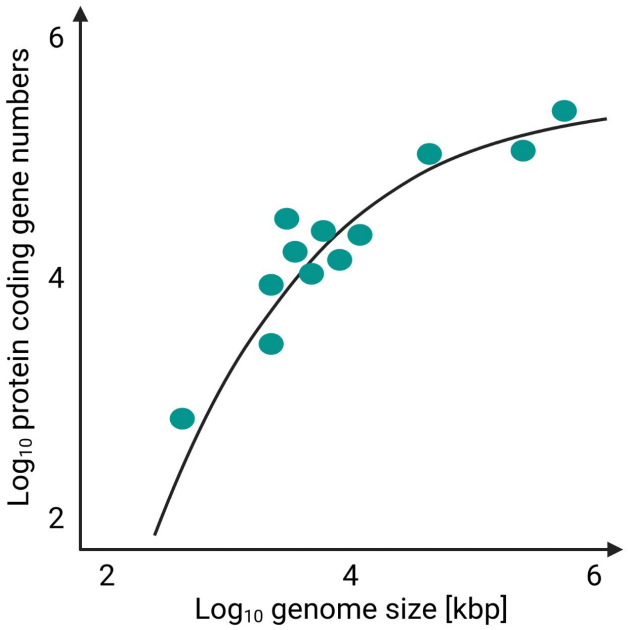


Finding genes in a genome sequence



Gene numbers

- Average number of genes in plants: 27200
- Gene number is not proportional to genome size

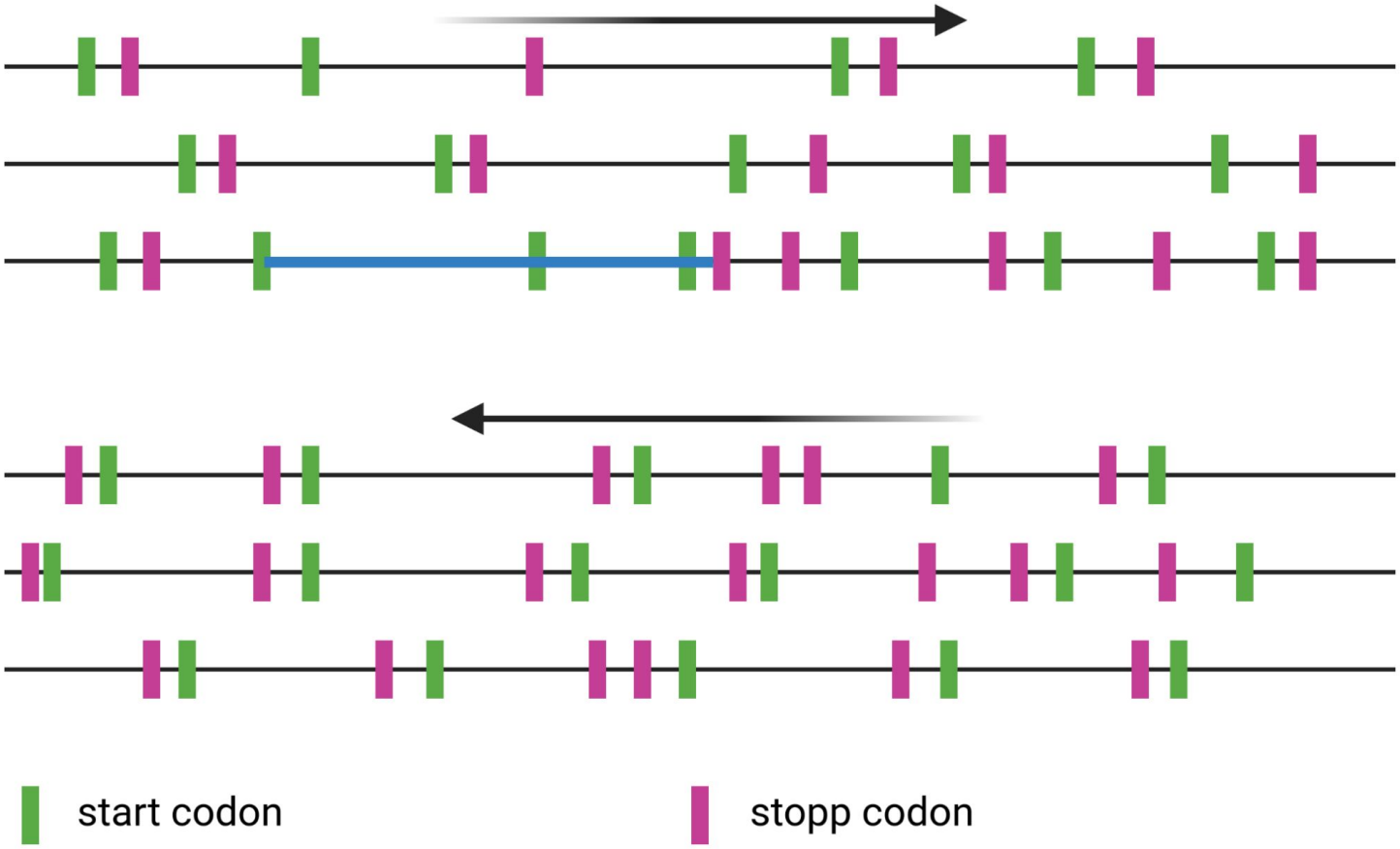


Pucker & Brockington, 2019: 10.1186/s12864-018-5360-z
Michael, 2014: 10.1093/bfgp/elu005

Repeat masking

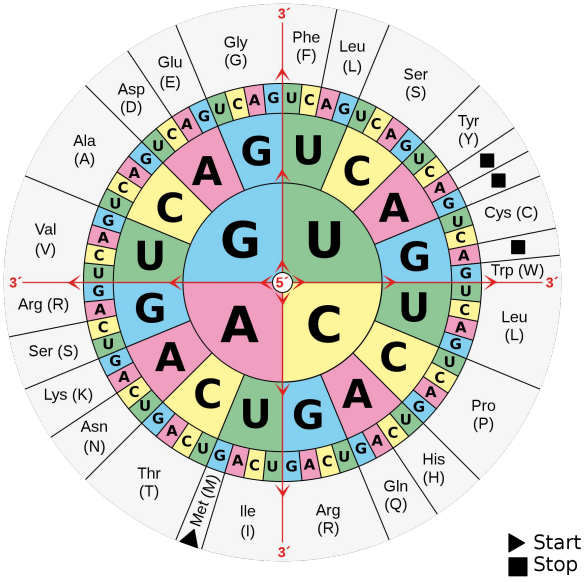
- Simple sequence repeats (SSR)
- Transposable elements (TE)
- Centromeric repeats (CEN)
- Telomeric repeats (TEL)

Finding ORFs



Codon usage

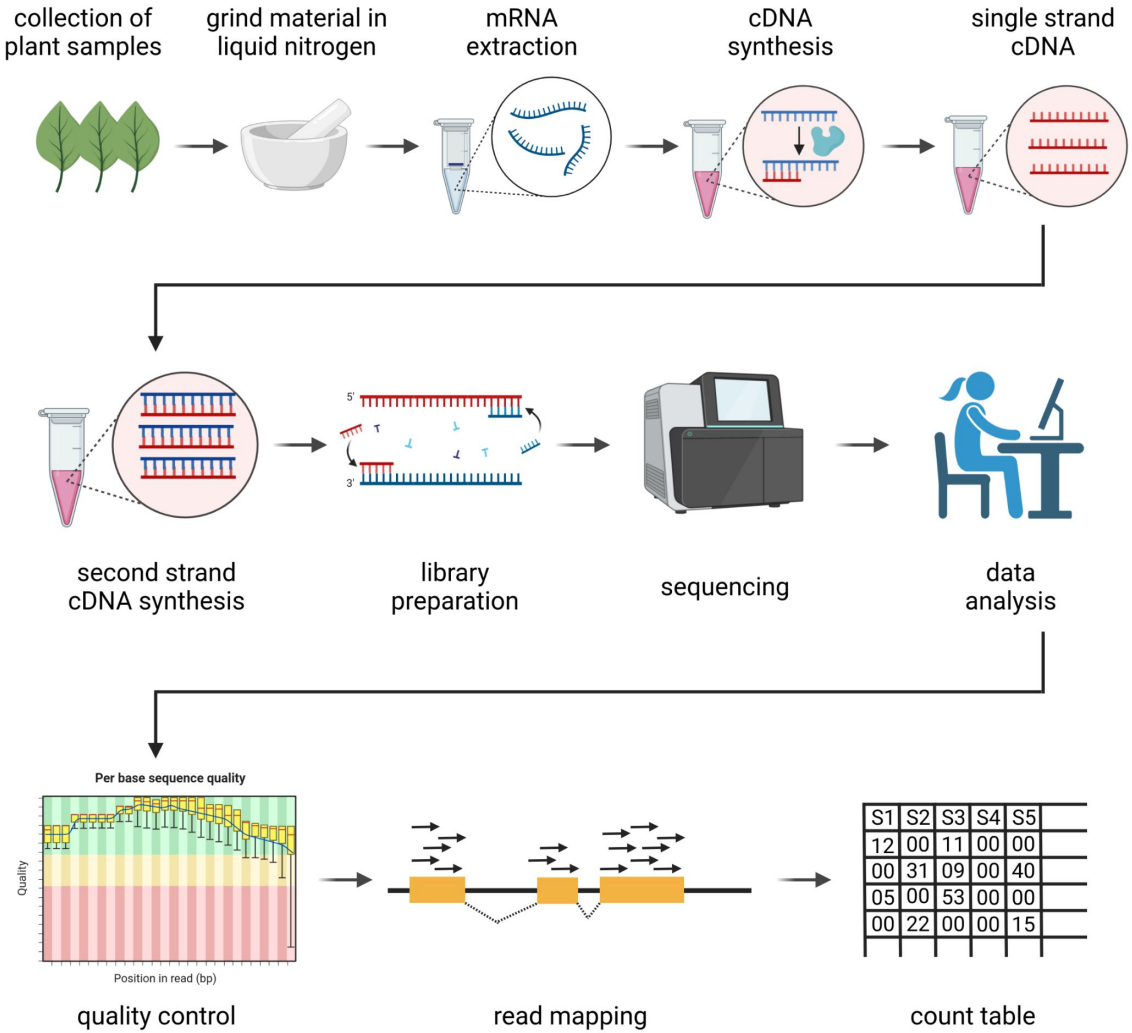
- Protein coding sequences have specific properties
- Codon usage is different between species i.e. different species prefer different codons for certain amino acids
- Rare codons slow down translation (useful between domains)
- Usage of dicodons (=hexamers)
- Codon usage can give additional CDS/ORF support



Parameters for *ab initio* gene prediction

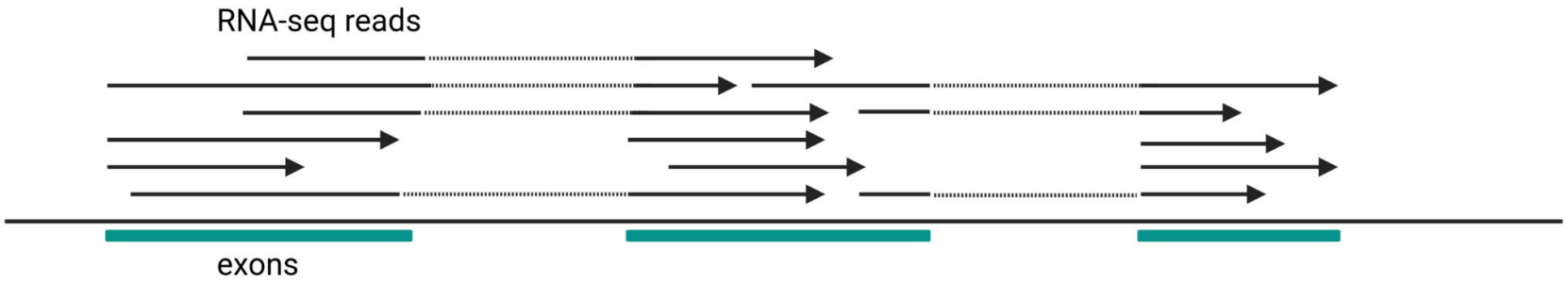
- Features: utr, exon, intron, intergenic region
- Composition of different features (dicodons)
- feature lengths
- Number of features per gene
- Possible splice sites

RNA-seq


collection of plant samples
grind material in liquid nitrogen
mRNA extraction
cDNA synthesis
single strand cDNA
second strand cDNA synthesis
library preparation
sequencing
data analysis
Per base sequence quality
Quality
Position in read (bp)
quality control
read mapping
S1 S2 S3 S4 S5
12 00 11 00 00
00 31 09 00 40
05 00 53 00 00
00 22 00 00 15
count table

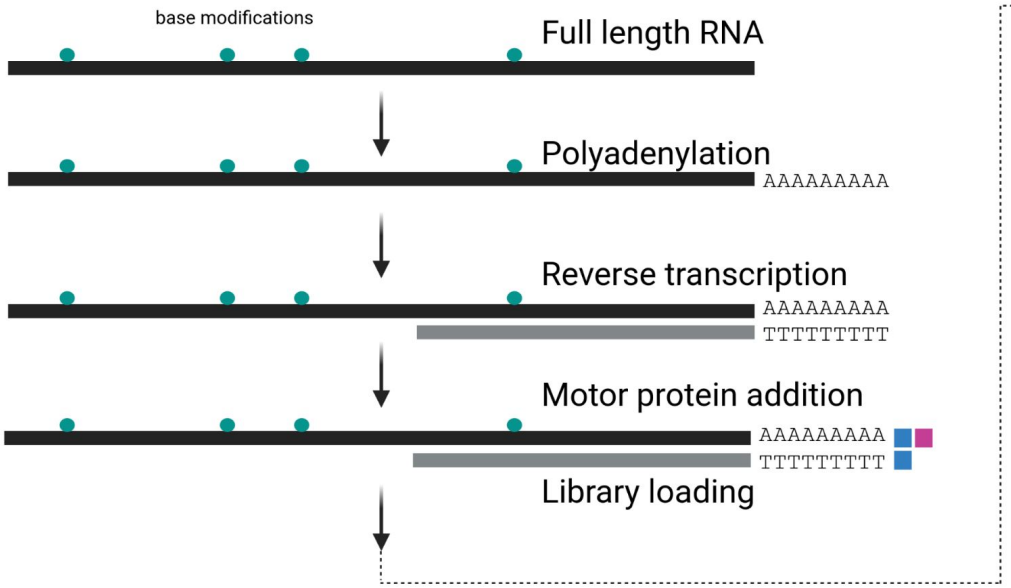
RNA-seq hints

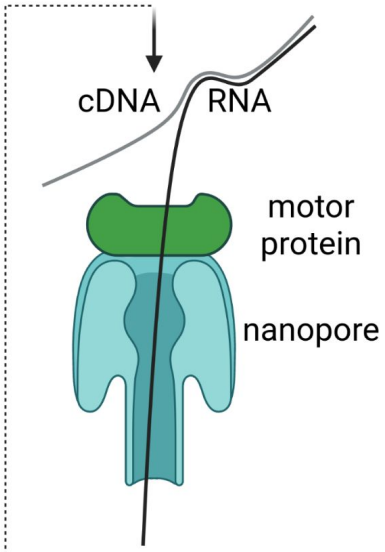
- Aligned RNA-seq reads indicate exon positions
- Splitting of reads indicates intron positions
- CDS can be identified as ORF within the covered regions



Full length transcript sequences

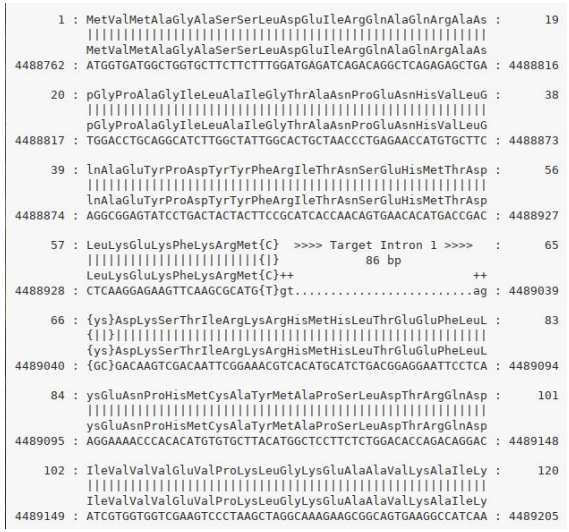
- Capturing full length transcripts for RNA-seq (Illumina)
- Full length cDNA sequencing (PacBio, ONT)
- Direct RNA sequencing or cDNA sequencing (ONT)





Proteins as hints (exonerate)

- Exonerate can align coding sequences/peptide sequences to DNA
- Intron positions are identified and intron information is given
- Exonerate can handle different types of splice sites



Annotation quality assessment

- BUSCO (Benchmarking Universal Single-Copy Orthologs)
  - Example: C:98%[S:85%,D13%],F:1.2%,M0.8%,n:1500
  - C = complete BUSCO genes
  - S = single copy BUSCO genes
  - D = duplicates BUSCO genes
  - F = fragmented BUSCO genes
  - M = missing BUSCO genes
  - n = number of BUSCO query sequences

- NLRome: assessment of high continuity genome sequences

Transposable elements (TEs)

- Transposons shape plant genomes (genome obesity)

- Systematics:
  - Class I (retrotransposons)
    - LTR: Copia, Gypsy, Bel-Pao, Retrovirus, ERV
    - DIRS: DIRS, Ngaro, VIPER
    - PLE: Penelope
    - LNE: R2, RTE, Jockey, L1, I
    - SINE: tRNA, 7SL, 5S
  - Class II (DNA transposons) - Subclass 1
    - TIR: Tc1-Mariner, hAT, Mutator, Merlin, Transib, P, PiggyBac, PIF-Harbinger, CACTA
    - Crypton
  - Class II (DNA transposons) - Subclass 2
    - Helitron
    - Maverick

Wicker et al., 2007: 10.1038/nrg2165

Annotation of TEs

- Benchmarking study of TE annotation tools: https://github.com/oushujun/EDTA

- RepeatMasker: https://www.repeatmasker.org/
  - Screens genomic sequence for TEs
  - Soft/hard masking of genomic sequence
  - Dfam and Repbase are important databases
    - Dfam: open collection of TE sequences (https://www.dfam.org/home)
    - Repbase: subscription-based collection (https://www.girinst.org/)
  - Different search engines can be used

- RepeatModeler2: http://www.repeatmasker.org/RepeatModeler
  - Pipeline for discovery of TEs

- Extensive *de novo* TE Annotator (EDTA): https://github.com/oushujun/EDTA
  - Complex pipeline for TE annotation

Ou et al., 2019: 10.1186/s13059-019-1905-y
Flynn et al., 2020: 10.1073/pnas.1921046117
Tarailo-Graovac & Chen, 2009: 10.1002/0471250953.bi0410s25

Sharing structural annotation

- Species specific databases:
  - TAIR: *Arabidopsis thaliana*
  - BananGenomeHub: *Musa acuminata*

- EMBL/EBI: European Nucleotide Archive of the European Bioinformatics Institute (https://www.ebi.ac.uk/ena/browser/home)

- PLAZA (https://bioinformatics.psb.ugent.be/plaza/)

- Phytozome (https://phytozome-next.jgi.doe.gov/)

Van Bel et al., 2022: 10.1093/nar/gkab1024

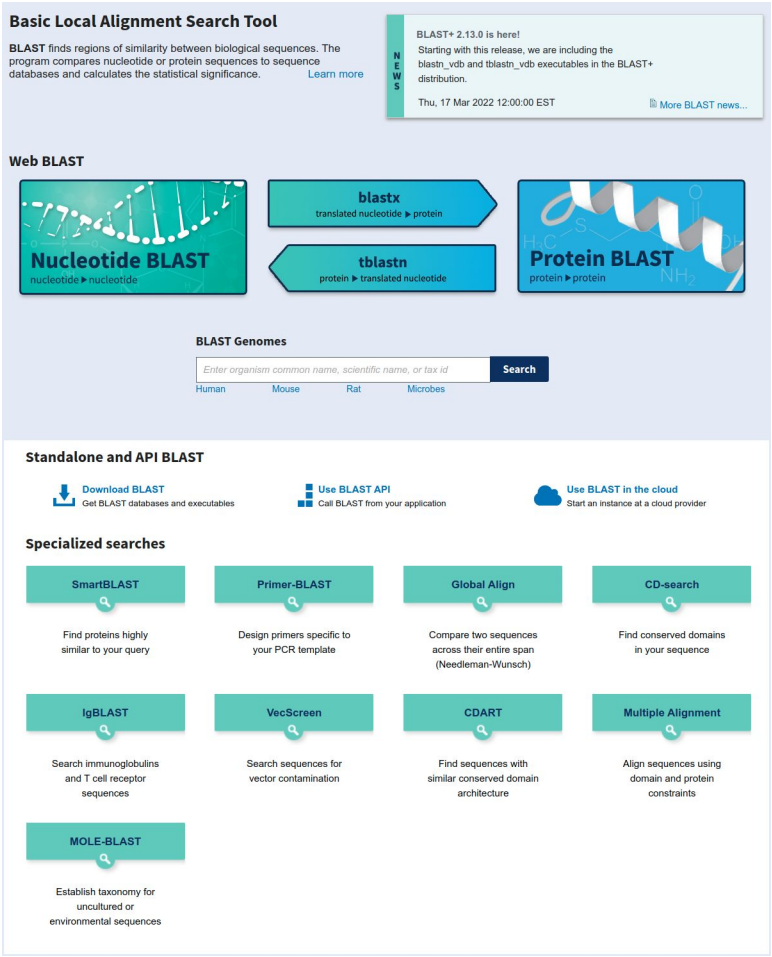
Functional annotation

- What is the function of a gene?
- Knockout experiments for all genes are time consuming and expensive
- Annotation transfer: orthologs are assumed to have the same function
- Tools:
  - BLAST: https://blast.ncbi.nlm.nih.gov/Blast.cgi
  - Pfam: https://pfam.xfam.org/
  - InterProScan5: http://www.ebi.ac.uk/interpro/search/sequence/
  - Shoot: https://github.com/davidemms/SHOOT
  - KEGG: https://www.genome.jp/kegg/
  - GO: http://geneontology.org/
  - MetaCyc: https://metacyc.org/
  - KIPEs: https://github.com/bpucker/KIPEs
  - BRENDA: https://www.brenda-enzymes.org/
  - Mercator: https://plabipd.de/portal/mercator4

Altschul et al., 1990: 10.1016/S0022-2836(05)80360-2
Mistry et al., 2021: 10.1093/nar/gkaa913
Jones et al., 2014: 10.1093/bioinformatics/btu031
Karp et al., 2002: 10.1093/nar/30.1.59
Emms & Kelly, 2022: 10.1186/s13059-022-02652-8
Kanehisa & Goto, 2000: 10.1093/nar/28.1.27
Ashburner et al., 2000: 10.1038/75556
Pucker et al., 2020: 10.3390/plants9091103
Schomburg et al., 2002: 10.1093/nar/30.1.47
Schwacke et al., 2019: 10.1016/j.molp.2019.01.003

BLAST: Basic Local Alignment Search Tool

- Probably the most famous website of the NCBI
- Comparison of sequences against a large database
- Similar sequences are likely to have similar functions (ideally orthologs)
- Numerous variants of the initial BLASTn were developed



https://blast.ncbi.nlm.nih.gov/Blast.cgi
Altschul et al., 1990: https://doi.org/10.1016/s0022-2836(05)80360-2

Pfam: Protein family database

- Assignment of protein functions based on Hidden Markov Models (HMMs)
- Sequences are screened based on HMM profile

Sequences are aligned

↓

Modelling of insertions/deletions

↓

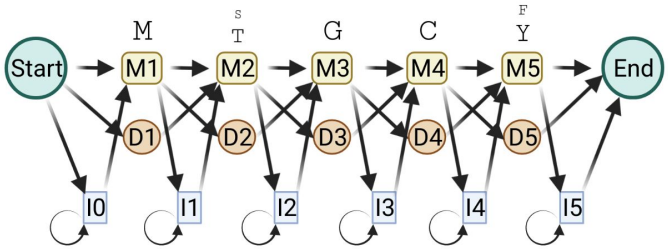
Amino acid frequency at each alignment position is encoded

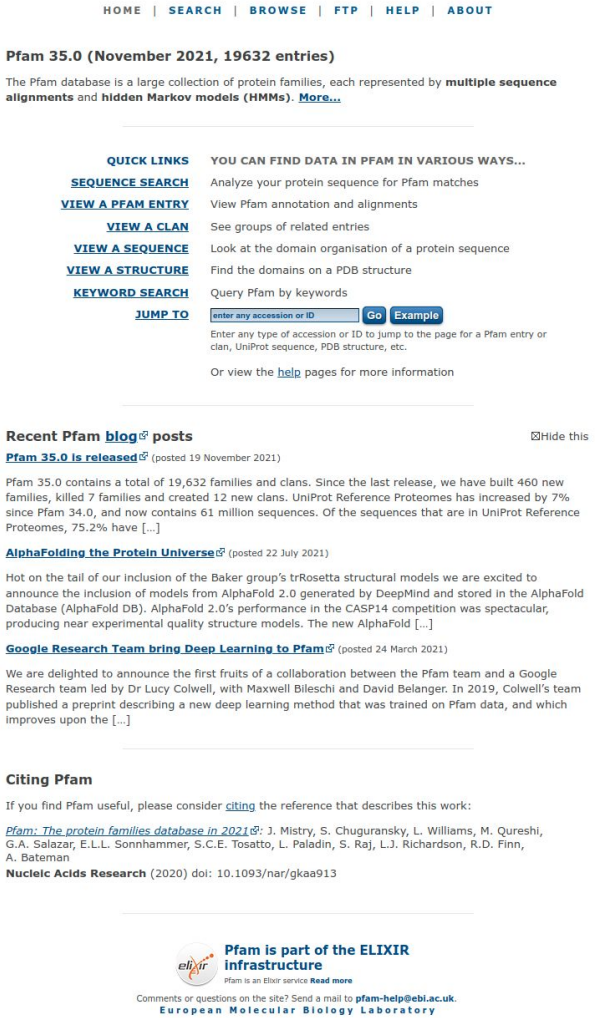
↓

HMM profile generated

```
seq1    MTGC-Y
seq2    MSGC-F
seq3    MTGC-Y
seq4    M-GCAY
        123456
```

2 = deletion
5 = insertion

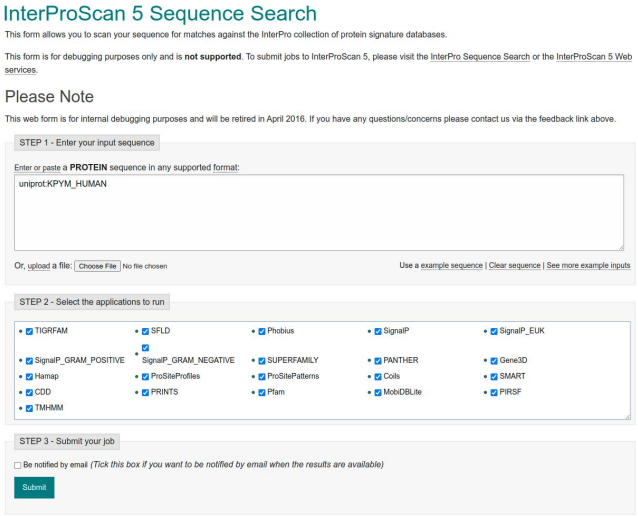




http://pfam.xfam.org/
Mistry et al., 2020: 10.1093/nar/gkaa913

InterProScan5

- Screen of protein sequences against collection of protein signatures
- Allows the assignment of functional annotation terms
- Available as web service, but also as stand alone tool



Results for job iprscan5-I20220320-102557-0980-87120812-p2m

Tool Output | Submission Details

Download in XML format | Download in TSV format | Download in GFF3 format | Download in SVG format | Download HTML tarball file | Download in JSON format

```
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   Gene3D   G3DSA:3.40.47.10          -       1     241     4.8E-101        T       20-03-2022
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   SUPERFAMILY   SSF53901          Thiolase-like   241     393     2.98E-51        T       20-03-20
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   SUPERFAMILY   SSF53901          Thiolase-like   10      237     2.38E-78        T       20-03-20
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   Pfam   PF02797 Chalcone and stilbene synthases, C-terminal domain      244     394     1.5E-71
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   PANTHER   PTHR11877:SF81   BNAA02G30320D PROTEIN   6       394     0.0     T       20-03-2022
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   Gene3D   G3DSA:3.40.47.10          -       242     395     9.7E-62 T       20-03-2022      IPR01603
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   PANTHER   PTHR11877       HYDROXYMETHYLGLUTARYL-COA SYNTHASE      6       394     0.0     T
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   CDD   cd00831 CHS_like          21      390     0.0     T       20-03-2022      -       -
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   PIRSF   PIRSF000451     PKS_III 7       394     0.0     T       20-03-2022      IPR011141
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   Pfam   PF00195 Chalcone and stilbene synthases, N-terminal domain      10      233     2.9E-119
sp|P13114|CHSY_ARATH   ba46b0f06bfbf1c91d161f0f91310f43   395   ProSitePatterns PS00441 Chalcone and stilbene synthases active site.    161     177     -
```

http://www.ebi.ac.uk/Tools/services/web/toolform.ebi?tool=iprscan5&sequence=uniprot:KPYM_HUMAN
Jones et al., 2014: 10.1093/bioinformatics/btu031

Shoot

- Initial search based on sequence similarity
- Phylogenetic relationships of sequences are considered based on a tree
- Universal tool, but computationally more intensive than a simple sequence similarity analysis

SHOOT.bio - the phylogenetic search engine

SHOOT is a phylogenetic alternative to BLAST. Instead of returning a list of similar sequences to a query sequence it returns a maximum likelihood phylogenetic tree with your query sequence embedded in it.

Try it out: https://shoot.bio/

Preprint: https://www.biorxiv.org/content/10.1101/2021.09.01.458564

Using the SHOOT command line tool

SHOOT allows you to search a protein sequence against a database of gene trees. It returns your gene grafted into the correct position within its corresponding gene tree.

Preparing a SHOOT phylogenetic database

0. Install dependencies:
   - Python libraries: ete3, sklearn, biopython
   - DIAMOND
   - MAFFT
   - EPA-ng & gappa (https://github.com/lczech/gappa)
   - Alternatively, IQ-TREE can be used instead of the combination EPA-ng + gappa
1. Run an OrthoFinder analysis on your chosen species, using the multiple sequence alginment option for tree inference, "-M msa".
   - Paper: Emms, D.M., Kelly, S. OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol 20, 238 (2019). https://doi.org/10.1186/s13059-019-1832-y
   - GitHub: https://github.com/davidemms/OrthoFinder
   - Tutorials: https://davidemms.github.io/
2. Run `python create_shoot_db.py RESULTS_DIRECTORY` , replacing "RESULTS_DIRECTORY" with the path to the OrthoFinder results directory from step 1.
3. Resolve polytomies (only necessary if using EPA-ng): `python bifurcating_trees.py RESULTS_DIRECTORY`

The OrthoFinder RESULTS_DIRECTORY is now a SHOOT database.

Running SHOOT

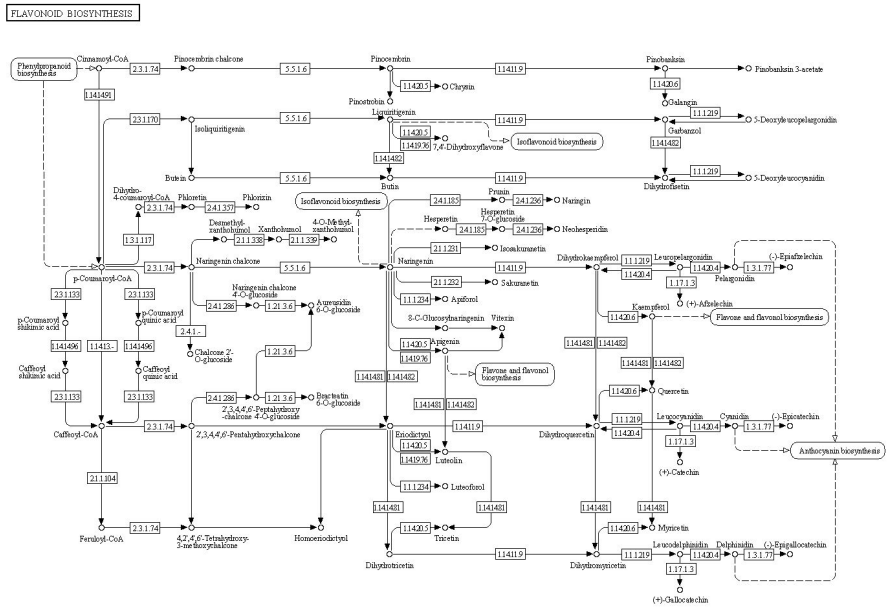
```
python shoot INPUT_FASTA SHOOT_DB
```

where INPUT_FASTA is a fasta file containing the amino acid sequence for the search and SHOOT_DB is the SHOOT database directory created using the steps above.

https://github.com/davidemms/SHOOT

KEGG: Kyoto Encyclopedia of Genes and Genomes

- Maps of pathways showing the individual reactions with catalyzing enzymes
- Information about genomes and genes
- Chemical details about enzymes, substrates, and products
- KEGG is financed through a subscription model (for FTP download), but website is freely accessible

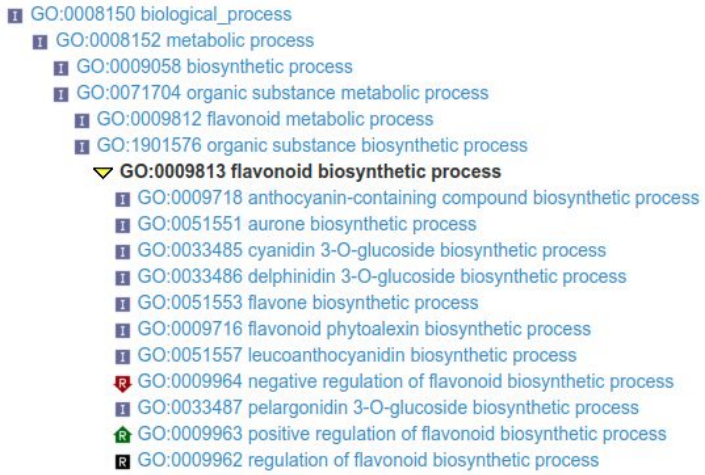


map00941 Flavonoid biosynthesis

Kanehisa & Goto, 2000: https://doi.org/10.1093/nar/28.1.27
Kanehisa et al., 2022: https://doi.org/10.1002/pro.4172
https://www.genome.jp/kegg/

Gene Ontology (GO)

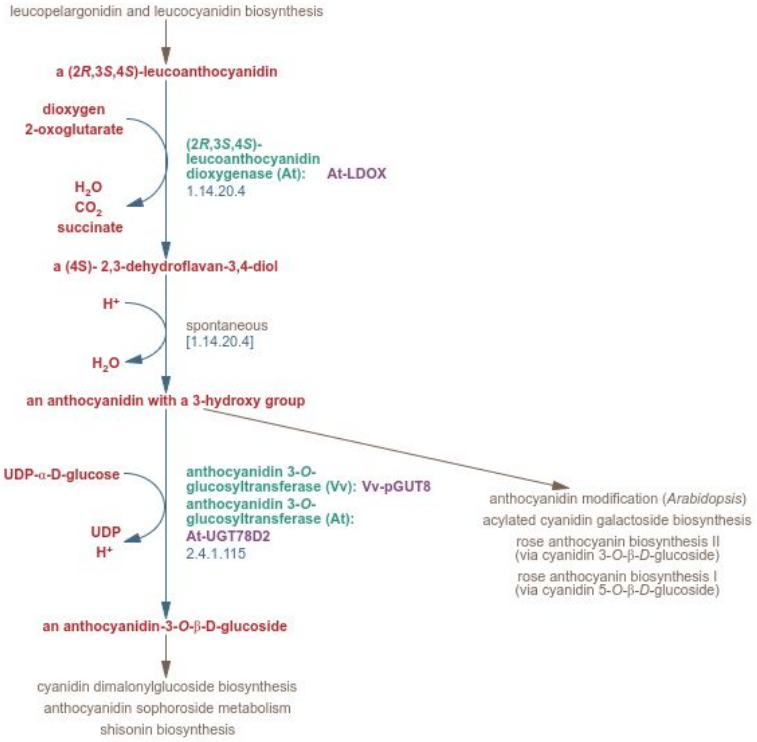
- Defined statements about the function of a gene (controlled vocabulary)
- Hierarchical structure
  - Example: ‘metabolic process’ > ‘biosynthetic process’ > … > ‘chalcone synthase’
- Supported by the Alliance of Genome Resources
- Connected with various other databases e.g. TAIR, FlyBase, Reactome, UniProt
- Machine readable to allow automatic processing
- Tools: Blast2GO and AmiGO
  - analyze the function of a sequence (web service and standalone)



http://amigo.geneontology.org/amigo/term/GO:0009813
The Gene Ontology Consortium, 2007: 10.1093/nar/gkm883

MetaCyc: Metabolite Encyclopedia

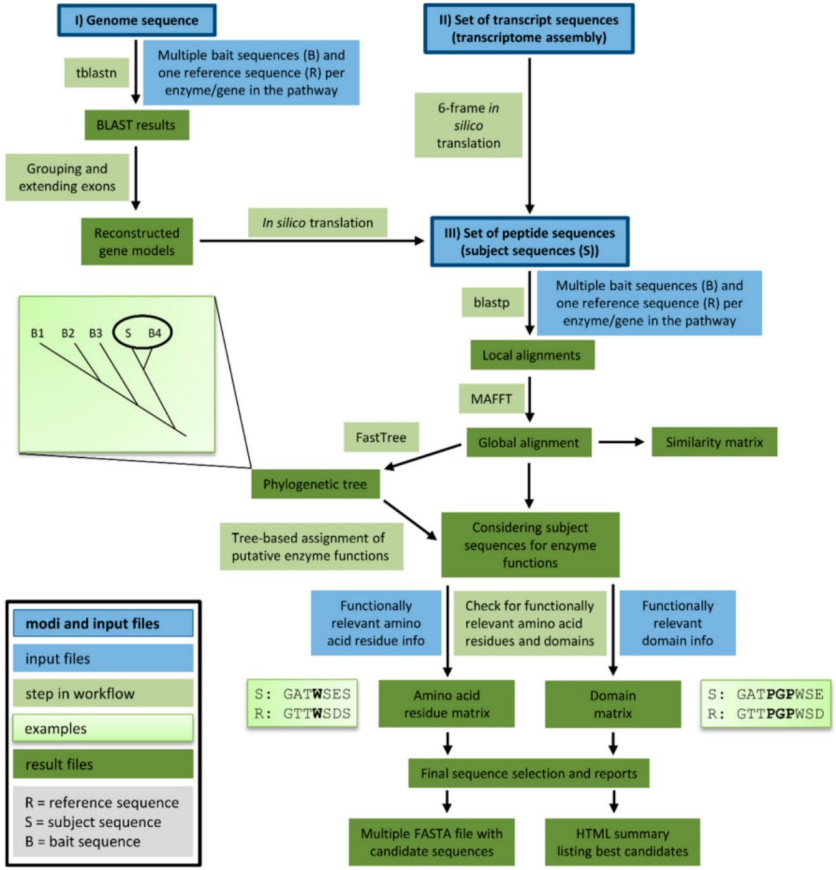
- Integrates genomic data with functional annotation
- Visualization of pathway databases
- Shows intermediates and enzymes of biosynthesis pathways
- MetaFlux: flux-balance analysis



https://metacyc.org/
Karp et al., 2015: https://doi.org/10.48550/arXiv.1510.03964
Figure: https://biocyc.org/META/NEW-IMAGE?type=PATHWAY&object=PWY-5125

KIPEs

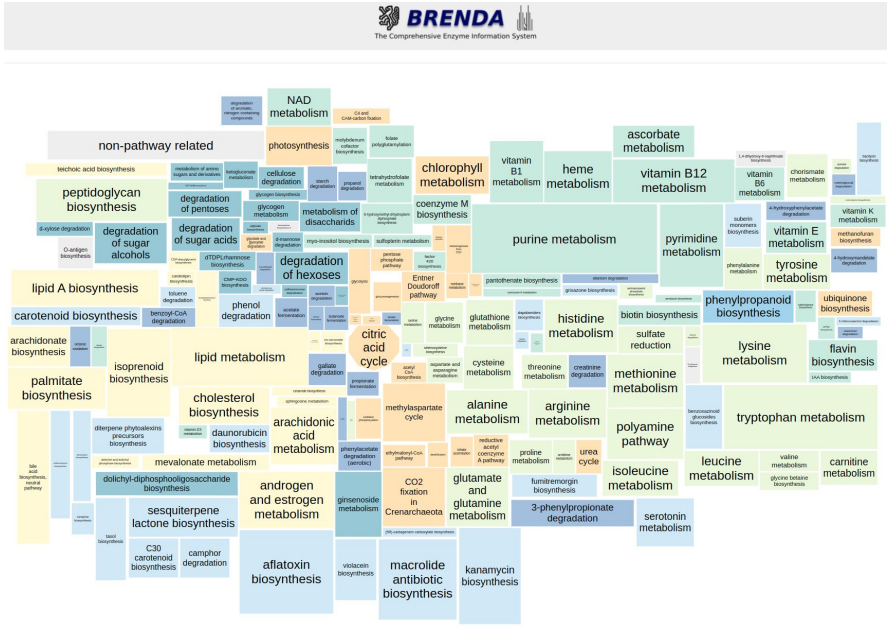
- Identification of all players in a biosynthesis pathway
- Dedicated to the identification of enzymes
- Functionally relevant amino acids need to be known
- Web server: http://pbb.bot.nat.tu-bs.de/KIPEs/



https://github.com/bpucker/KIPEs
Pucker et al., 2020: 10.3390/plants9091103

BRENDA: BRaunschweig ENzyme DAtabase

- Enzyme database hosted at TU Braunschweig (BRICS)
- Text and structure-based queries
- Visualization of pathways
- Manual curation of datasets
- Many details about enzyme properties (substrates, kinetics, mutants, …)



www.brenda-enzymes.org
Chang et al., 2021: 10.1093/nar/gkaa1025

Mercator - functional annotation of protein sequences

- Online tool for the annotation of all protein sequences in a submitted FASTA file

- FASTA format:
  - Header line starts with '>' followed by the sequence name
  - Header is followed by unrestricted number of sequence lines

```
>TRINITY_DN100013_c0_g1_i1
MIGPMPGMEGKLMLPGASPVGLEVVLSLVVASTPILSDTSLCTPSFYHFLLLLGPITSISNLIVRPFFLTLLSSITVIYGRLCFFAFDFYVY
>TRINITY_DN100016_c0_g1_i1
MPKKSSNIQQNNQNRIKRKGRPPKKKKYVQQIDSSDEDILSVRHASTRPRIISIRRNEIPMRPEIHIPPTSIPSFNNPNNIHQSSSDRQMPP
>TRINITY_DN100019_c0_g1_i1
MPPKAADKKPAAKAPATASKAPEKKDAGKKTAATGDKKKRTKARKETYSSYIYKVLKQVHPDTGISNRAMSILNSFVNDIFERVATEASKLA
>TRINITY_DN10001_c0_g1_i2
MAKVGNPIVDIETDGSVNEPESSEKNIEVSSSSTQAPESTNTTELLVNEKKAFSLATPAVRRVAREHNIDINNIKGTGKNGRITKEDILNYV
>TRINITY_DN100025_c0_g1_i1
MVENQDGCFFKPGWKEFVRSNDLEGGDFLVFNLVDKISYQVVIFDGTCCPKDLCPFSIMNPIFQHLRNKIFLSKKEEIKLKGNRKVHSVNEN
>TRINITY_DN10002_c0_g1_i1
MSDELNQVFQRCREQKRPVFVAFVTAGYPDSEETVDILLGLEAGGADIIELGIPFTDPMVDGKTIQDANNVALENKIDISKCLSYVSESRAK
```

https://plabipd.de/portal/mercator-sequence-annotation
Lohse et al., 2014: 10.1111/pce.12231
Haak et al., 2018: 10.3389/fmolb.2018.00062

Summary

- Assembly problem & assembly challenges
- Coverage
- Assembly evaluation - CCC
- Scaffolding with genetic linkage information
- Finding genes with/without hints
- Tools for functional annotation

Time for questions!

Questions

1. What are contigs, scaffolds, and pseudochromosomes?
2. What are coverage depth and coverage extent?
3. What are challenges during the assembly process?
4. What are the three ‘C’s of assembly evaluation?
5. What is the N50?
6. What is BUSCO? / How does it work?
7. What options exist to improve long read assemblies?
8. What are different features in a eukaryotic gene?
9. What types of repeats in plant genomes do you know?
10. How does RNA-seq work?
11. What are potential hints for a gene prediction process?
12. Which tools can be used to assign function predictions to genes?