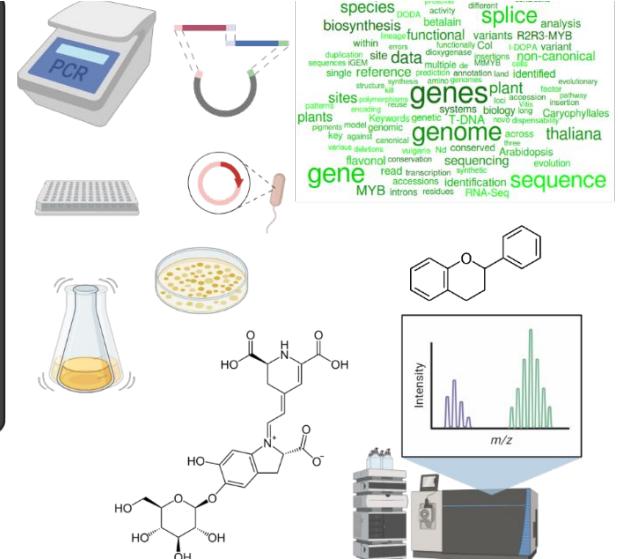
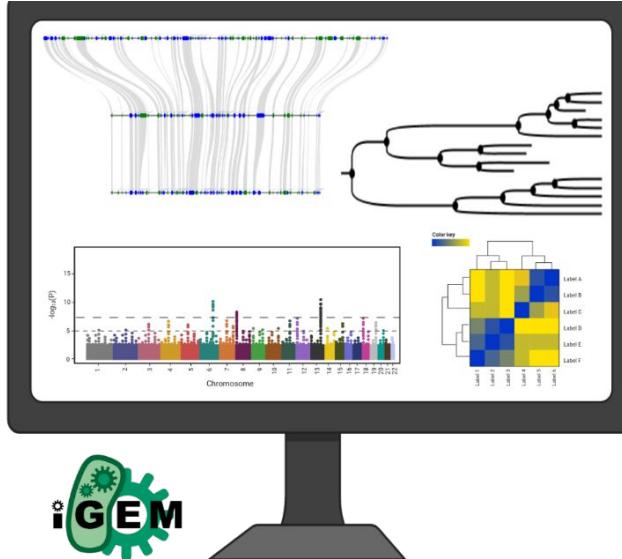
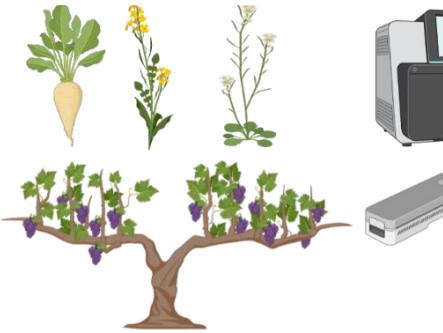




Technische
Universität
Braunschweig



Assembly and annotation of a genome sequence

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

Availability of slides

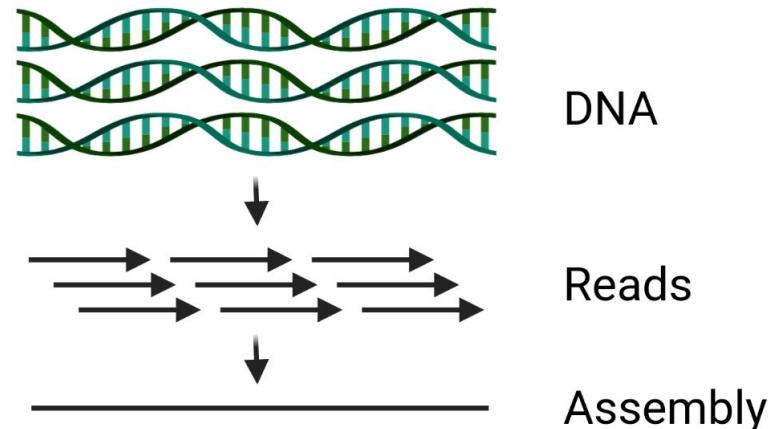
- All materials are freely available (CC BY) - after the lectures:
 - StudIP: Data Literacy in Genomics
 - GitHub: <https://github.com/bpucker/teaching>
- Questions: Feel free to ask at any time
- Feedback, comments, or questions: b.pucker[a]tu-bs.de

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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



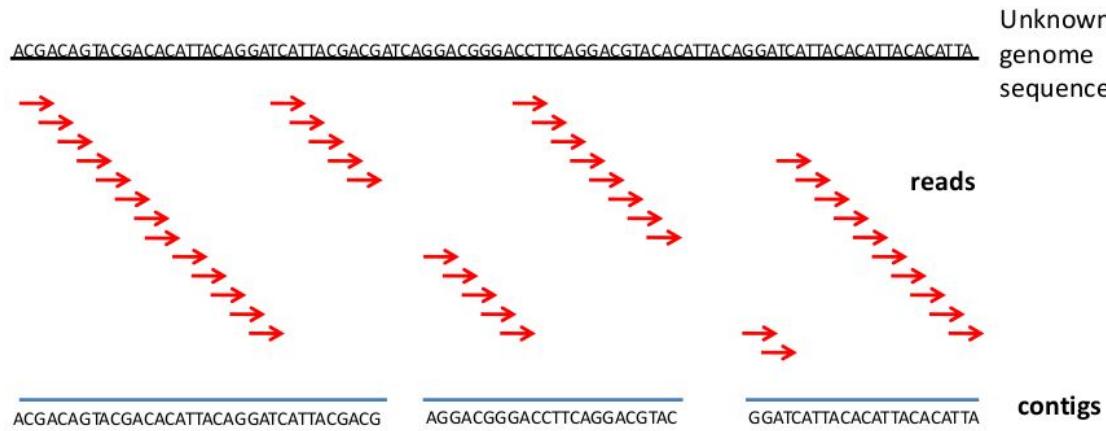
```
>TRINITY_DN100016_c0_g1_i1
MPKKSSNIQNNQNRIKRKGRPPKKKYVQQIDSSDEDILSVRHASTRPRIISIRRNEIPMRPEIHI
>TRINITY_DN100019_c0_g1_i1
MPPKAADKKPAAKAPATASKAPEKKDAGKKTAAATGDKKRTKARKETYSSYIYVLKQVHPDTGISN
>TRINITY_DN10001_c0_g1_i2
MAKVGNPIVDIETDGSVNEPESSEKNIEVSSSSTQAPESTNTTELLVNEKKAFSLATPAVRVAREH
>TRINITY_DN100025_c0_g1_i1
MVENQDGCCFKPGWKEFVRSNDLEGDFLVNLVDKISYQVVIFDGTCCPKDLCPSIMNPIFQHLR
>TRINITY_DN10002_c0_g1_i1
MSDELNQVFQRCREQKRPVFVAFTAGYPDSEETVDILLGLEAGGADIIELGIPFTDPMVDGKTIQD.
>TRINITY_DN100061_c0_g1_i1
MQQVVAKLKAIITKTNVTNENSPVENSSSTSATSSINNSLHGDLSRVFDNMELESNVSNSSISSNI
```



What is a contig/scaffold?



Contigs, scaffolds, pseudochromosomes

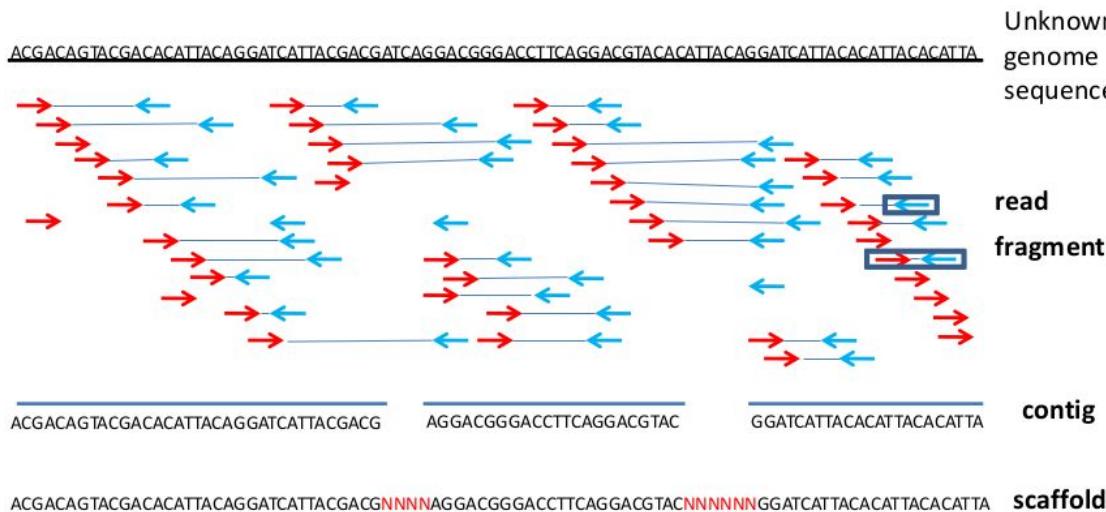


Contig = continuous sequence

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

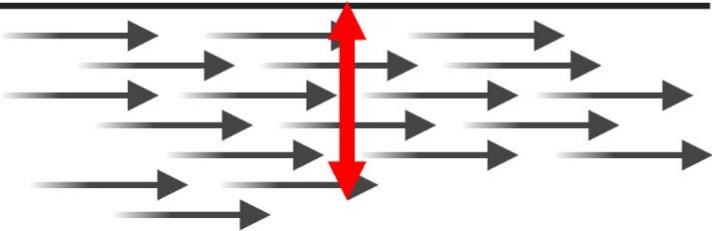
Gaps = regions between contigs that are represented by Ns

Pseudochromosomes = scaffolds representing an entire chromosome

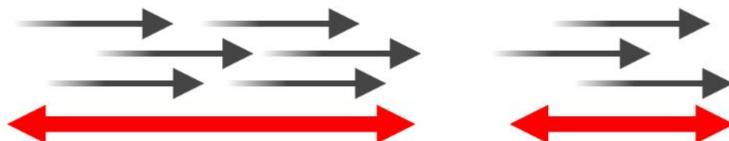


Sequencing coverage depth vs. coverage extent

Sequencing coverage depth



Sequencing 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 $d = N \times L / G$
- Coverage (depth) reflects total amount of sequencing data
- Coverage (depth) is very important parameter for sequencing projects

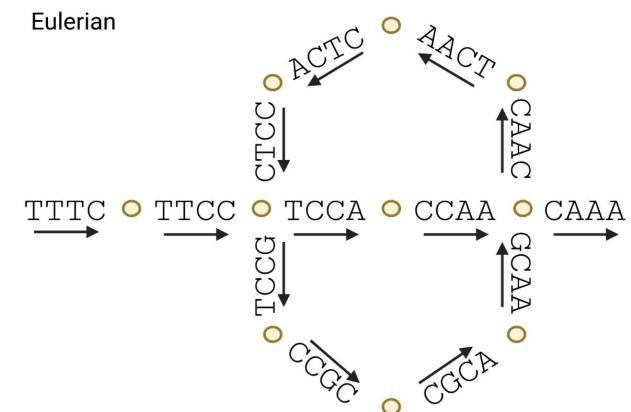
Sequencing coverage extent

- 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 Bruijn 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

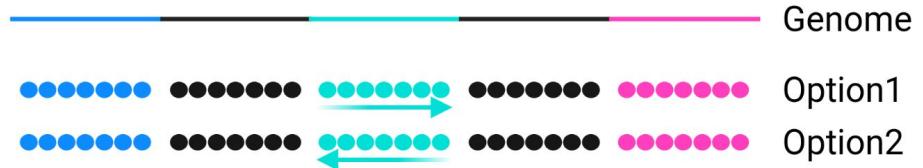
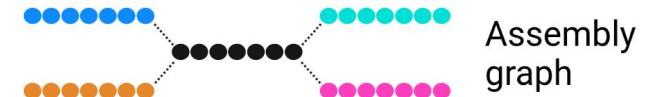


K-mer size

- 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



Miss-assembly:

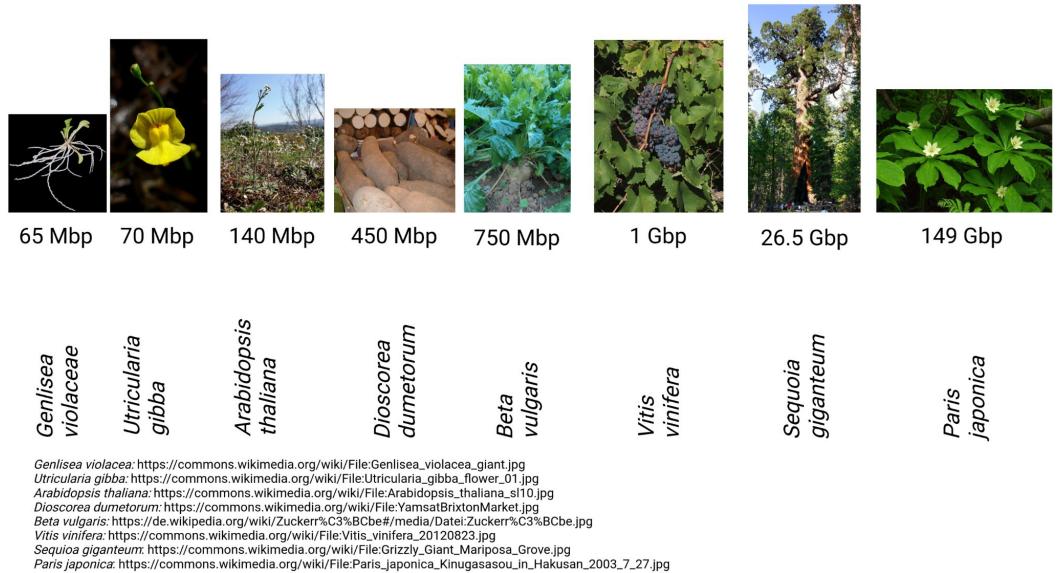


Correct assembly:

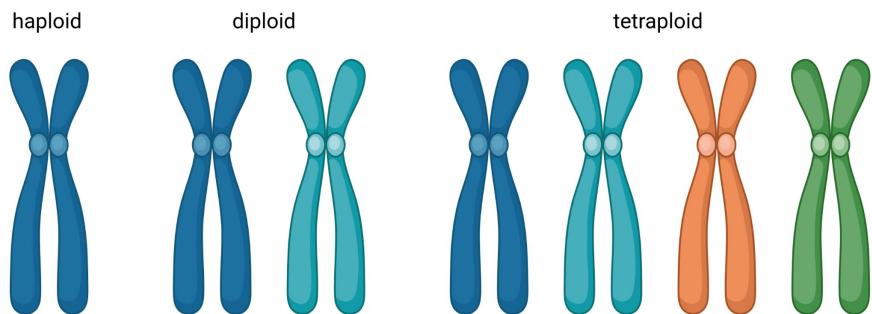


Assembly challenges (2)

- Genome size: variation from 65 Mbp to 149 Gbp



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



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

Assembly polishing

ONT assembly . . . ACGTTACGGTACGACATGACGTAAAAAAAACGATAGTACGTGTTAGCGTACGTACGTAACGTT . . .

Illumina reads

GACGTAAAAAAA - CGATAGTACGTGTTAGCGTACGTAC
ATGACGTAAAAAAA - CGATAGTACGTGTT
CGTAAAAAAA - CGATAGTACGTGTTAGCGTAC
ACATGACGTAAAAAAA - CGATAGTACGT
CGTAAAAAAA - CGATAGTACGTGTTAGCGT
GACGTAAAAAAA - CGATAGTA

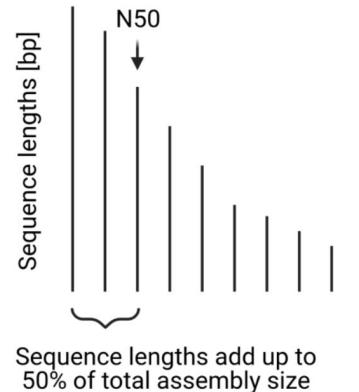
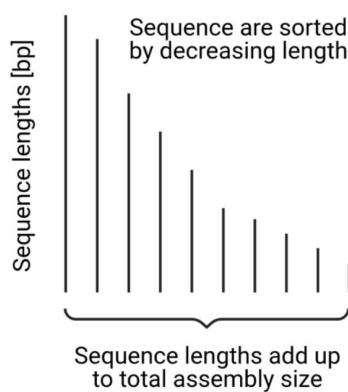
Assembly evaluation

- 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



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

BUSCO

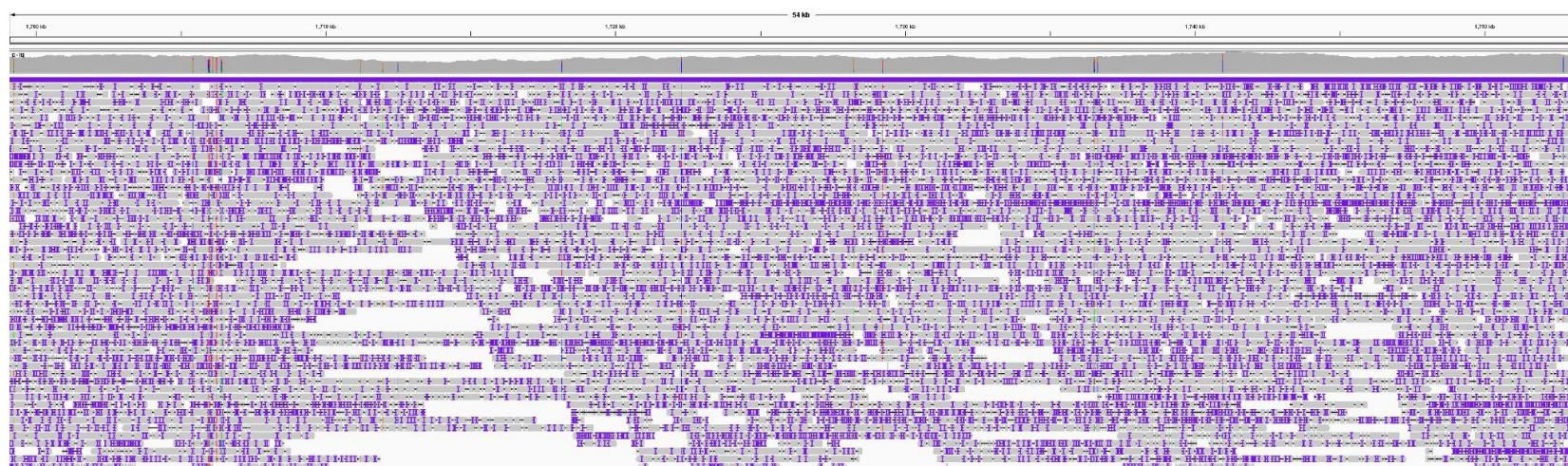
from QC to gene prediction and phylogenomics

BUSCO v5.3.2 is the current stable version!

[Gitlab](#), a [Conda package](#) and [Docker container](#) are also available.

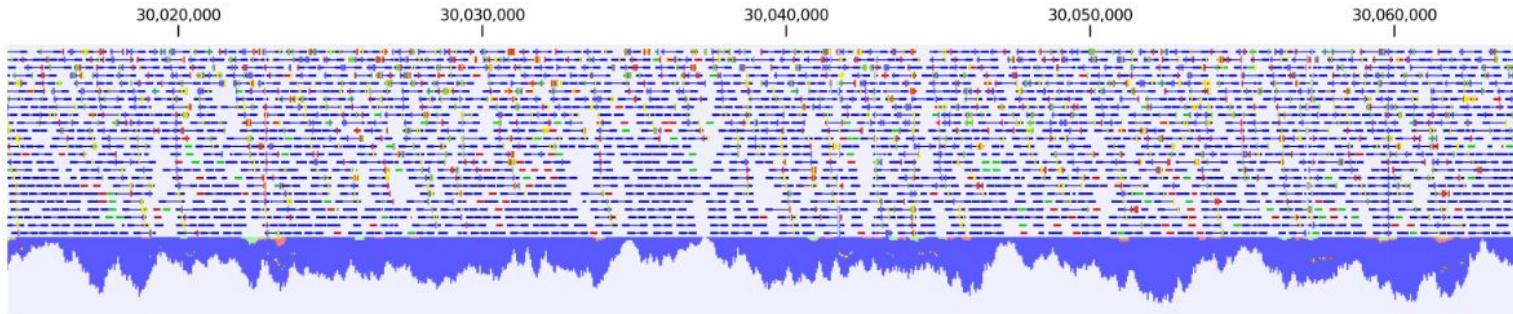
Evaluation: assembly correctness

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

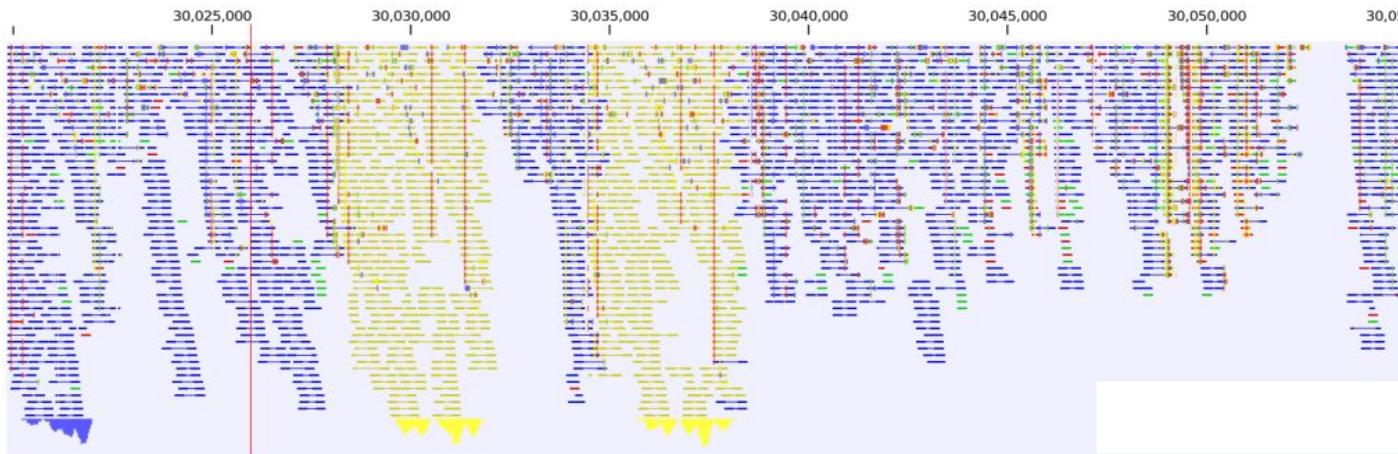


IGV: Thorvaldsdottir et al., 2013: [10.1093/bib/bbs017](https://doi.org/10.1093/bib/bbs017)
REAPR: Hunt et al., 2013: [10.1186/gb-2013-14-5-r47](https://doi.org/10.1186/gb-2013-14-5-r47)
SQUAT: Yang et al., 2019: [10.1186/s12864-019-5445-3](https://doi.org/10.1186/s12864-019-5445-3)

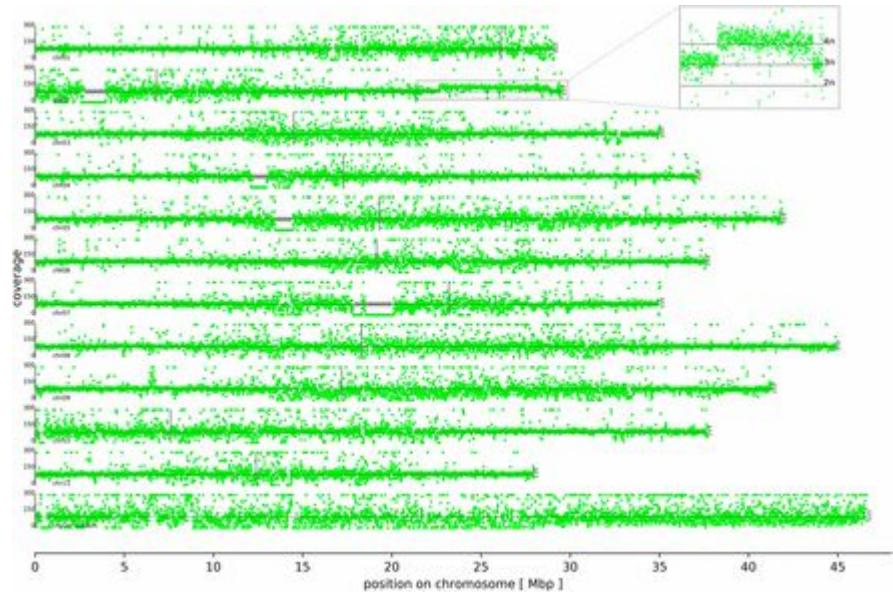
Read mappings (1)



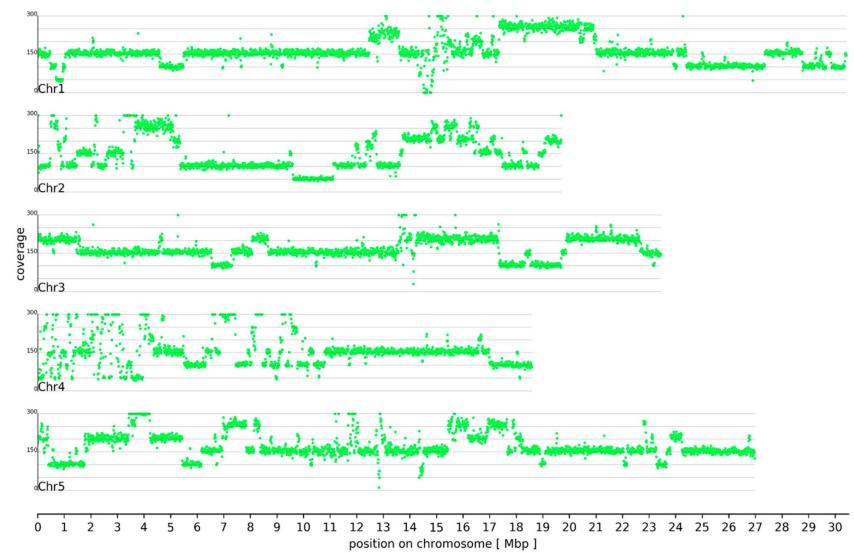
Coverage is too high to show all individual fragments at some positions



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

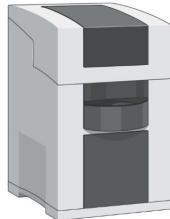
Integration of genetic linkage information (1)

- 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: CATAGAGAGAGATGAC
C: CATAGAGAGAGATGAC
D: CATAGAGAGAGAGAGATGAC
E: CATAGAGAGAGAGATGAC
F: CATAGGAGAGAGAGAGATGAC
G: ACATAGAGATGAC

Length analysis via capillary electrophoresis



CAPS: Cleaved Amplified Polymorphic Sequences

A:
B:
C:

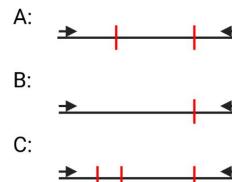
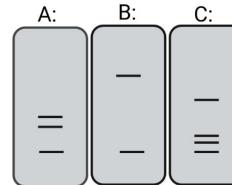
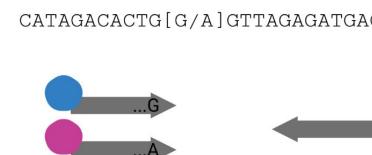


Diagram illustrating CAPS analysis. Three DNA strands (A, B, C) are shown with restriction enzyme cleavage sites indicated by red vertical lines and arrows pointing from the strand to the cut site. Strand A has one cut, strand B has two cuts, and strand C has three cuts.

PCR & restriction digest



KASP: Kompetitive Allele Specific PCR



qPCR with fluorescently labeled primers

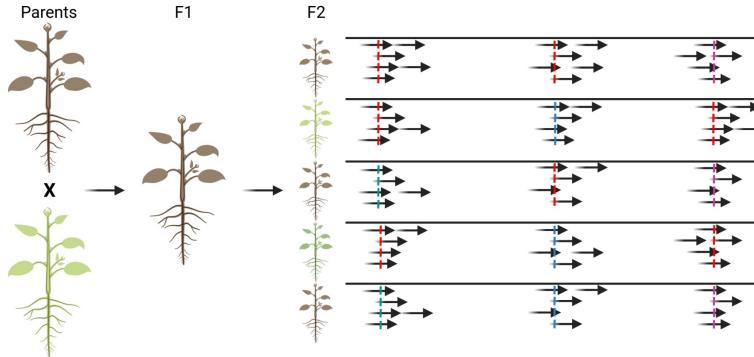


SSR: Holtgräwe et al., 2020: 10.3389/fpls.2020.00156
CAPS: Konieczny & Ausubel, 1993: 10.1046/j.1365-313x.1993.04020403.x
KASP: He et al., 2014: 10.1007/978-1-4939-0446-4_7

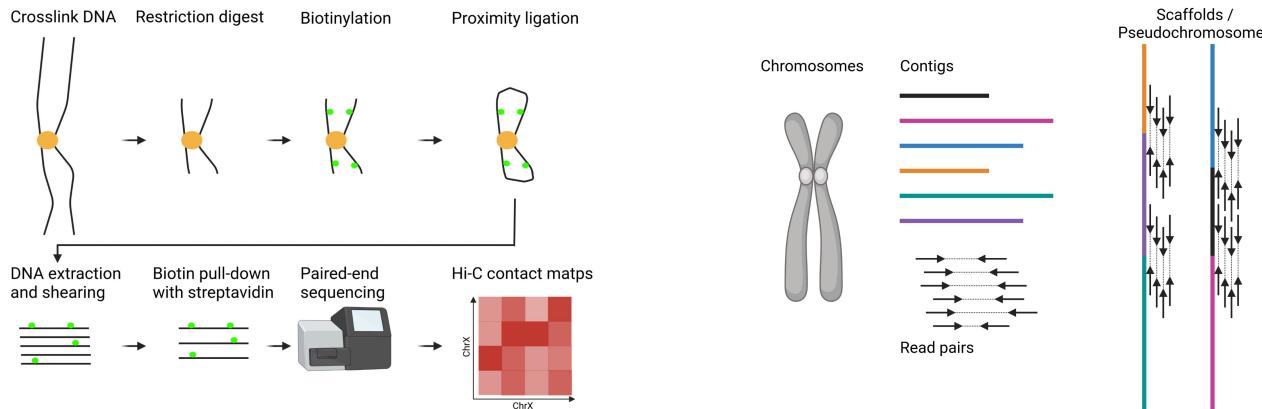


Integration of genetic linkage information (2)

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



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

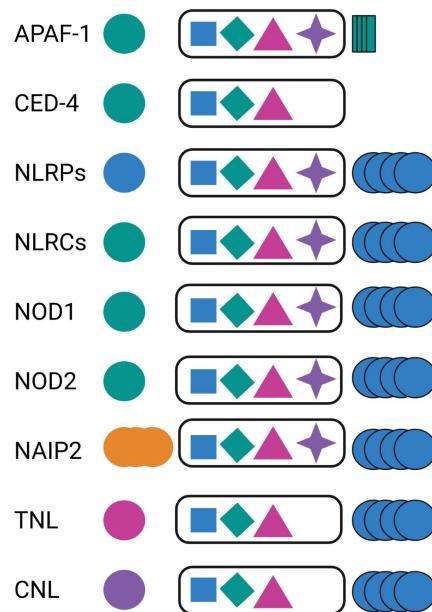


Checking challenging regions

- 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

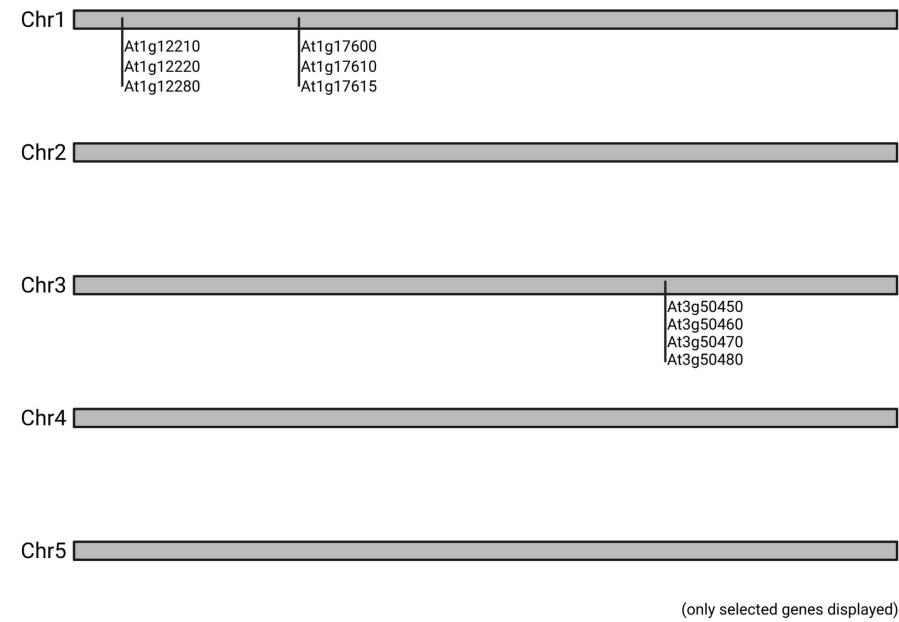
- NLR genes (NLRome) are often clustered in tandem repeat arrays
- NLR gene clusters are particularly tricky to assemble
- NLRome used for benchmarking high quality assembly



Signalling domains
CARD PYD BIR TIR CC

NOD sub-domains
NBD HD1 WHD HD2

C-terminal repeat domains
WD40 LRR

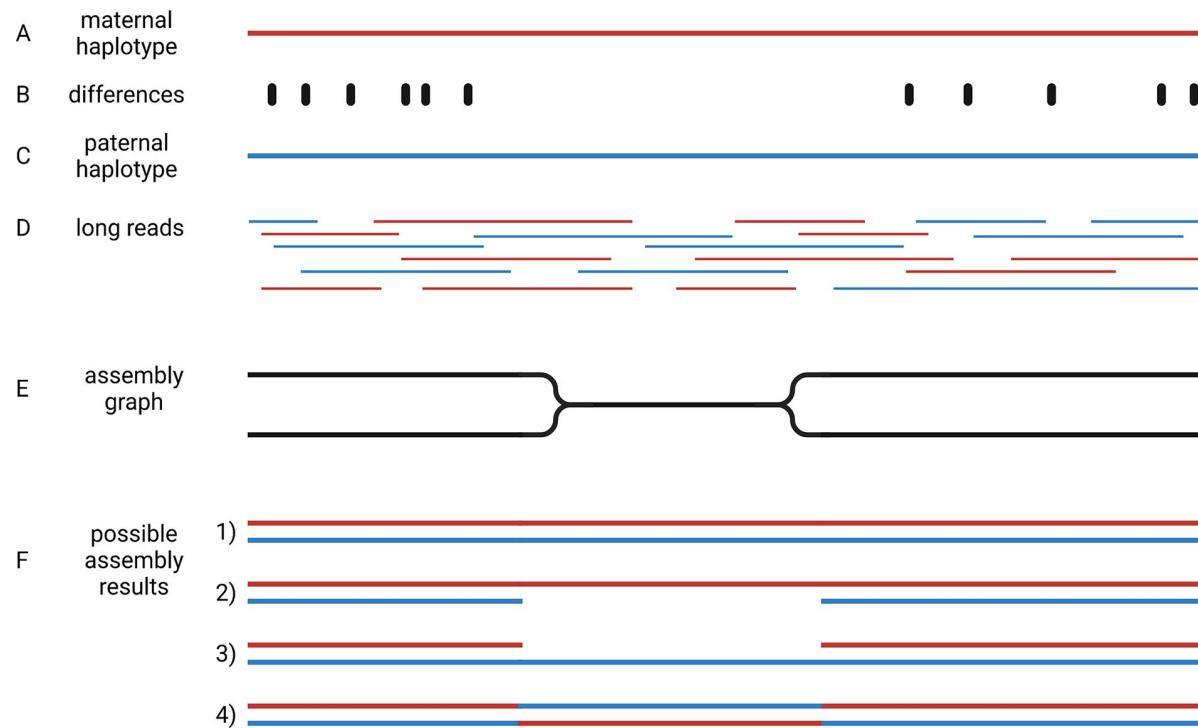


Van de Weyer et al., 2019: 10.1101/j.cell.2019.07.038



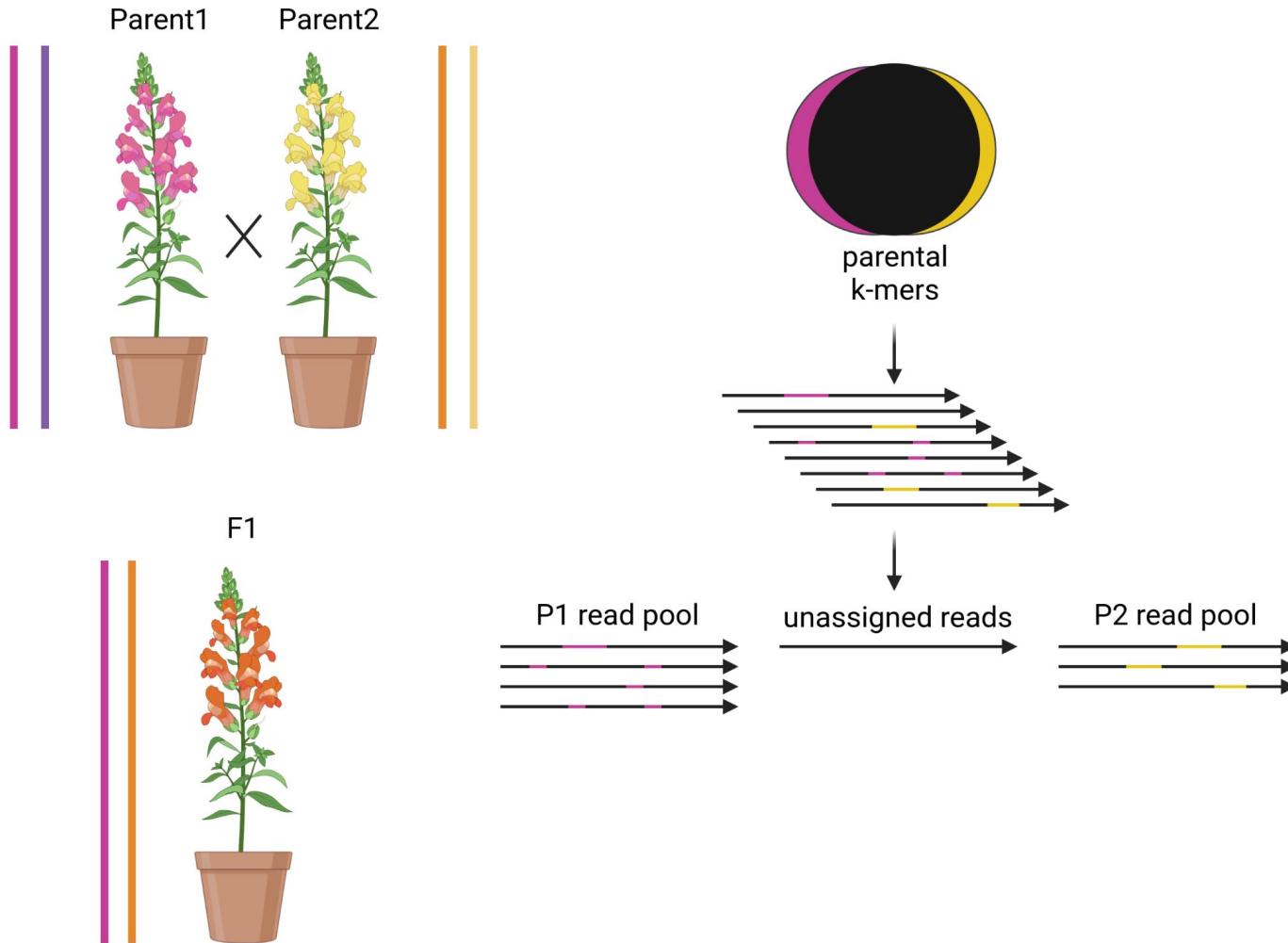
Haplophases

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



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

TrioBinning



Genome sequence annotation

Finding genes in a genome sequence

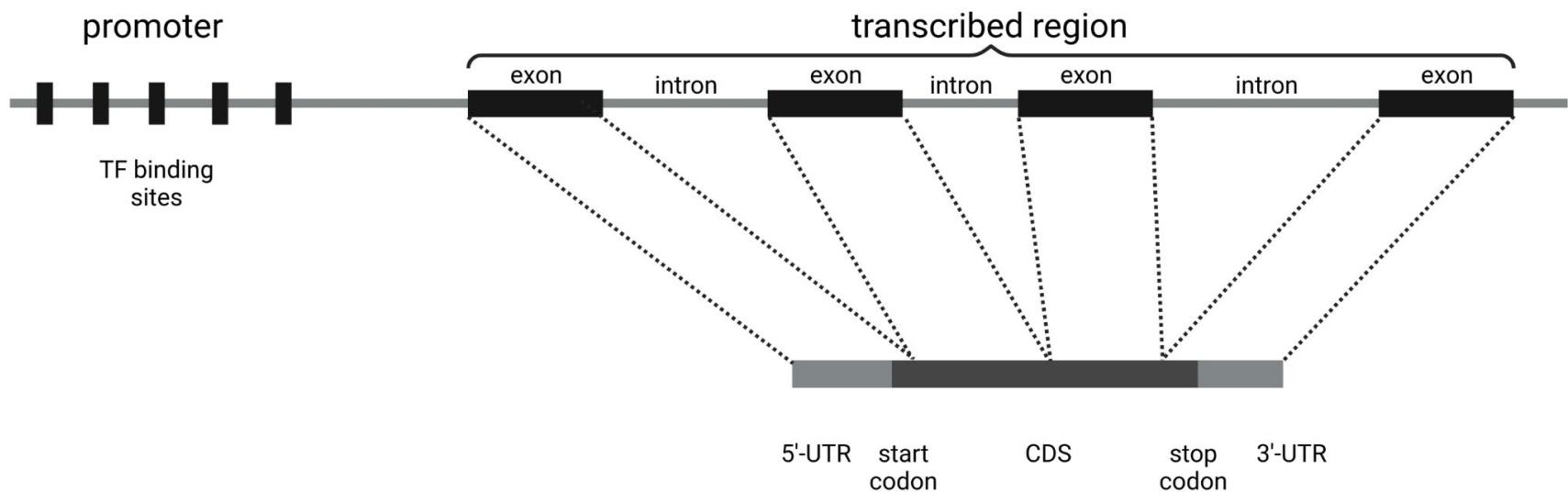
Plant gene structure

CDS = (Protein) Coding Sequence

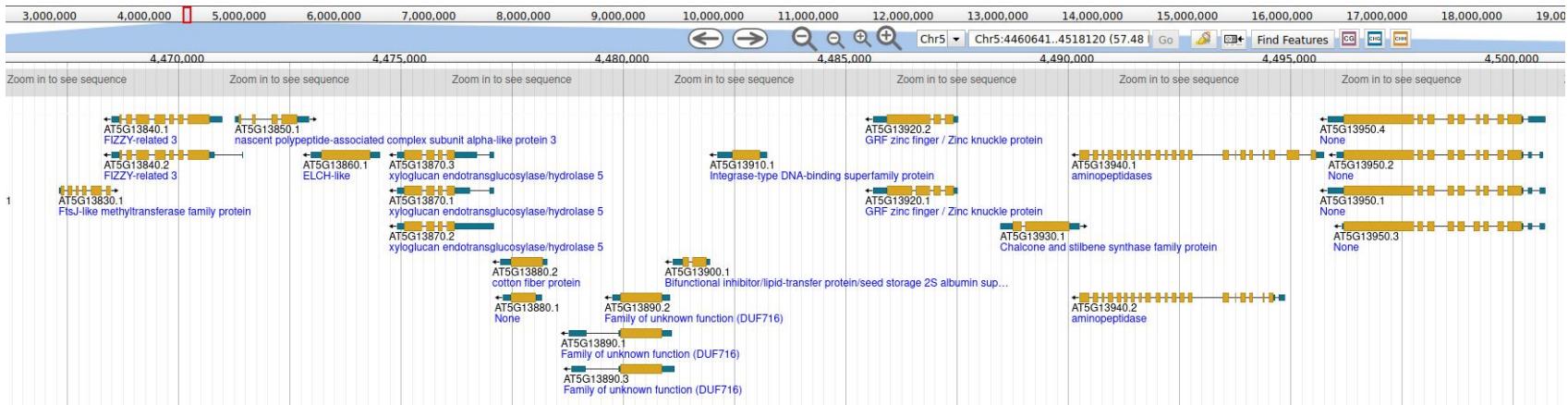
ORF = Open Reading Frame

UTR = UnTranslated Region

TF = Transcription Factor

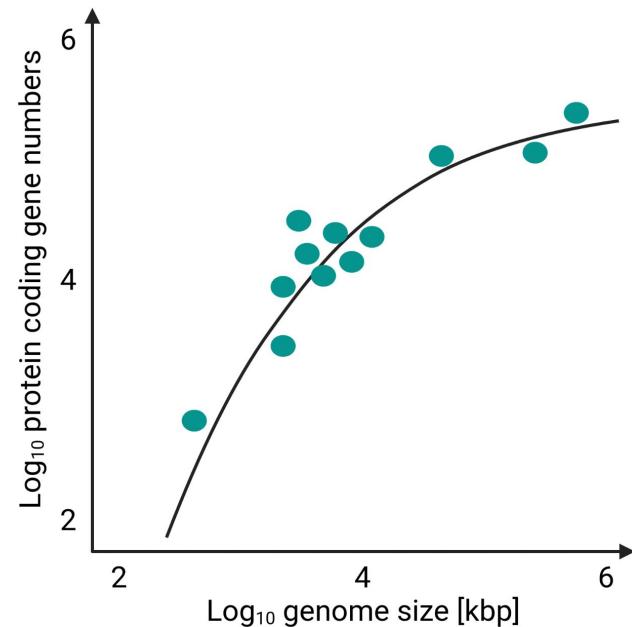


Finding genes in a genome sequence



Gene numbers

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

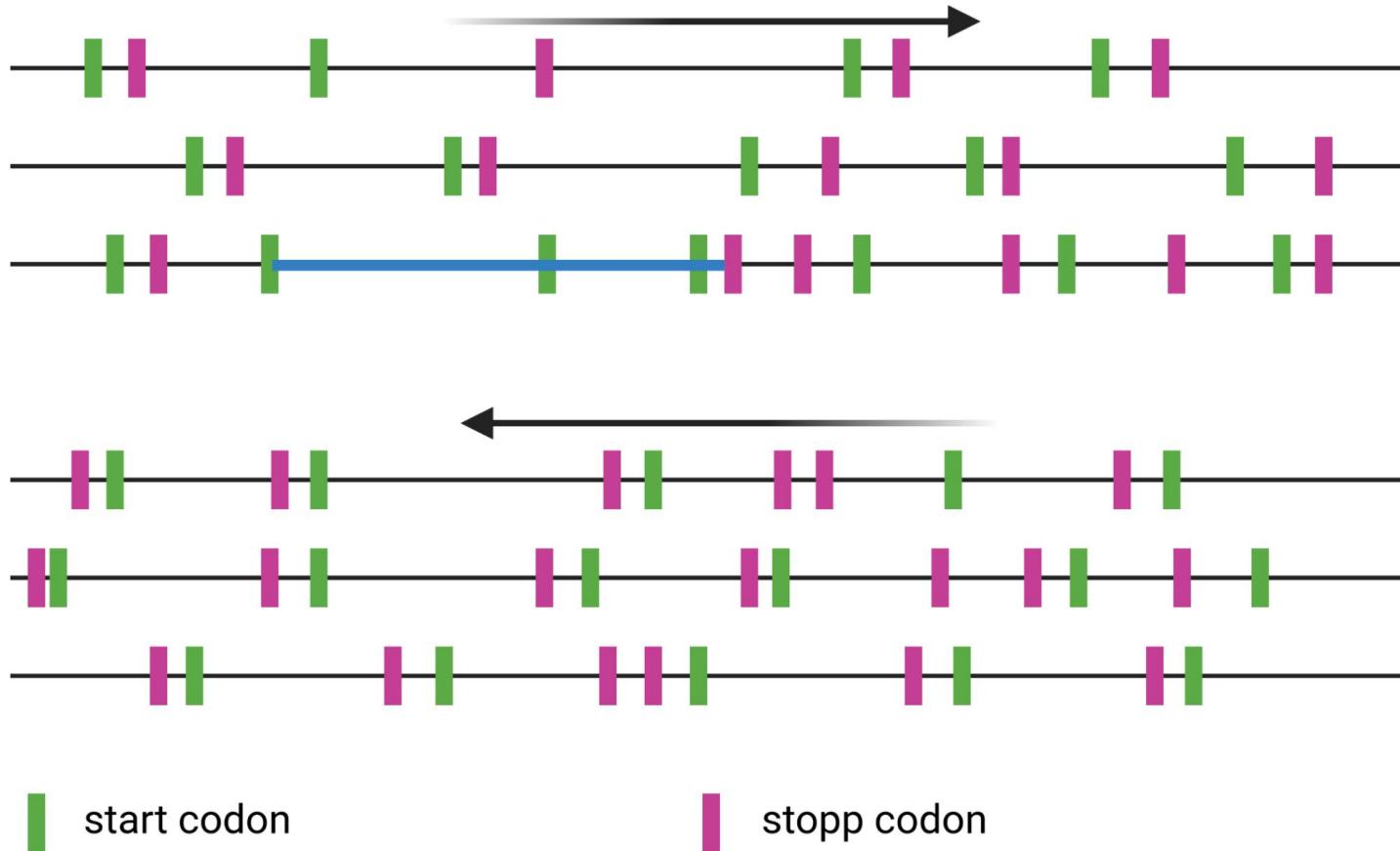


Pucker & Brockington, 2019: 10.1186/s12864-018-5360-z
Michael, 2014: 10.1093/bfgp/elu005

Repeat masking

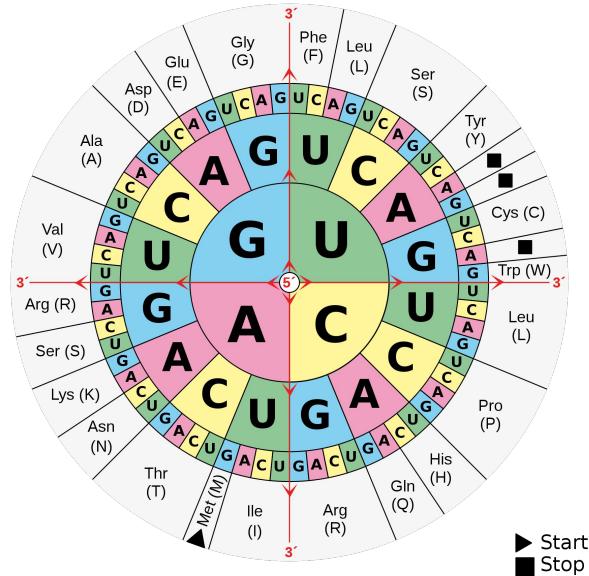
- Simple sequence repeats (SSR)
- Transposable elements (TE)
- Centromeric repeats (CEN)
- Telomeric repeats (TEL)

Finding ORFs



Codon usage

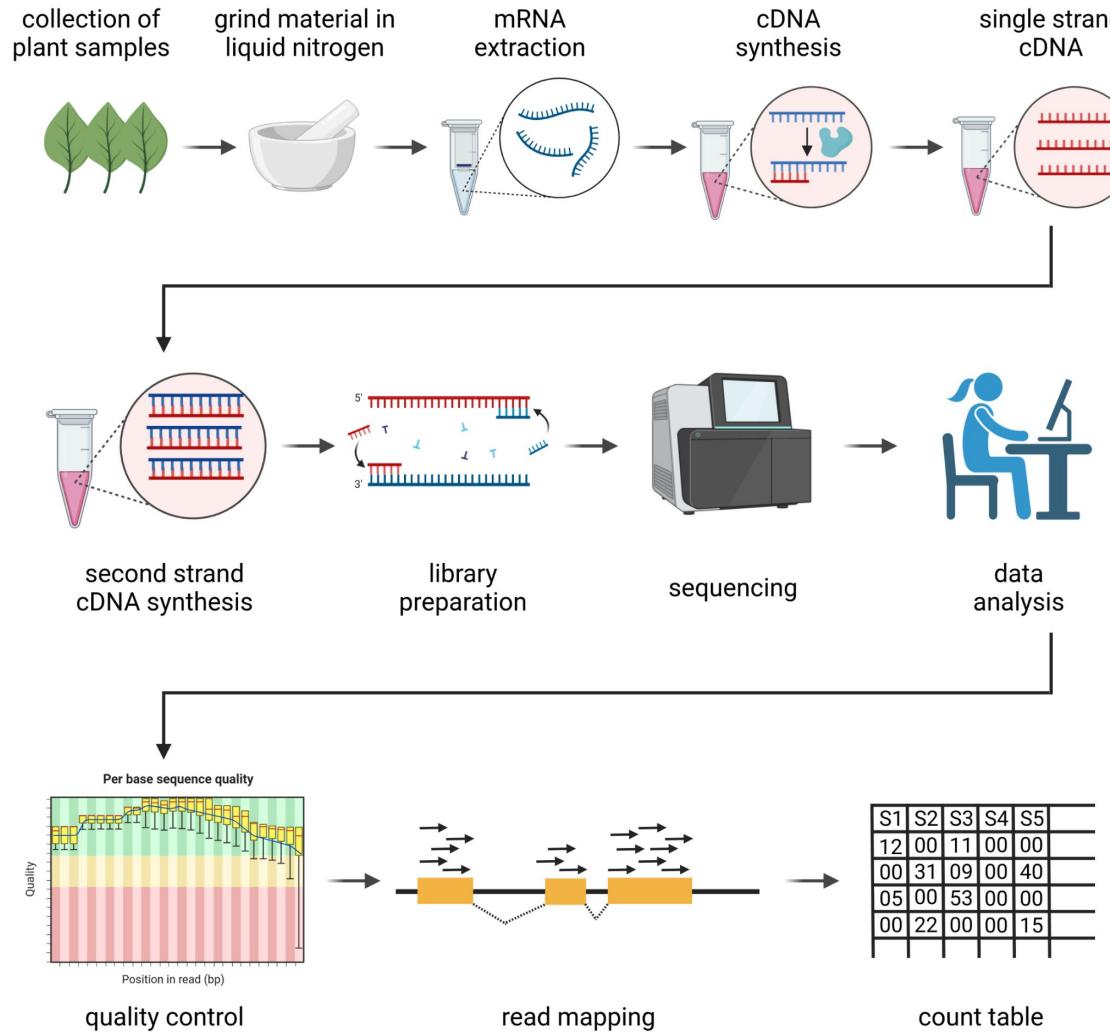
- Protein coding sequences have specific properties
 - Codon usage is different between species i.e. different species prefer different codons for certain amino acids
 - Rare codons slow down translation (useful between domains)
 - Usage of dicodons (=hexamers)
 - Codon usage can give additional CDS/ORF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



Parameters for *ab initio* gene prediction

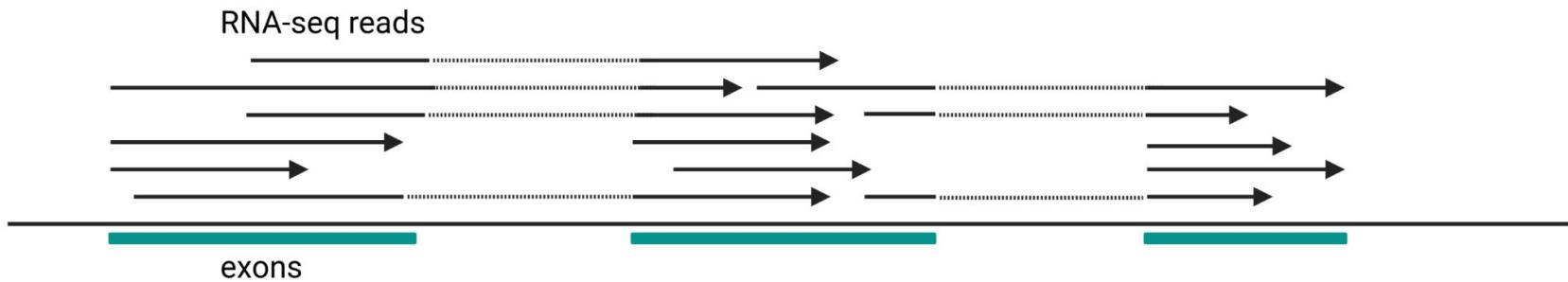
- Features: utr, exon, intron, intergenic region
- Composition of different features (dicodons)
- feature lengths
- Number of features per gene
- Possible splice sites

RNA-seq



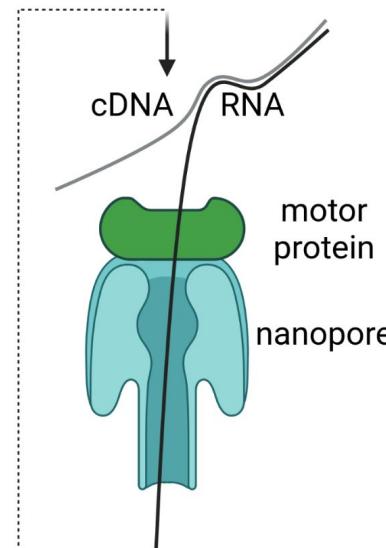
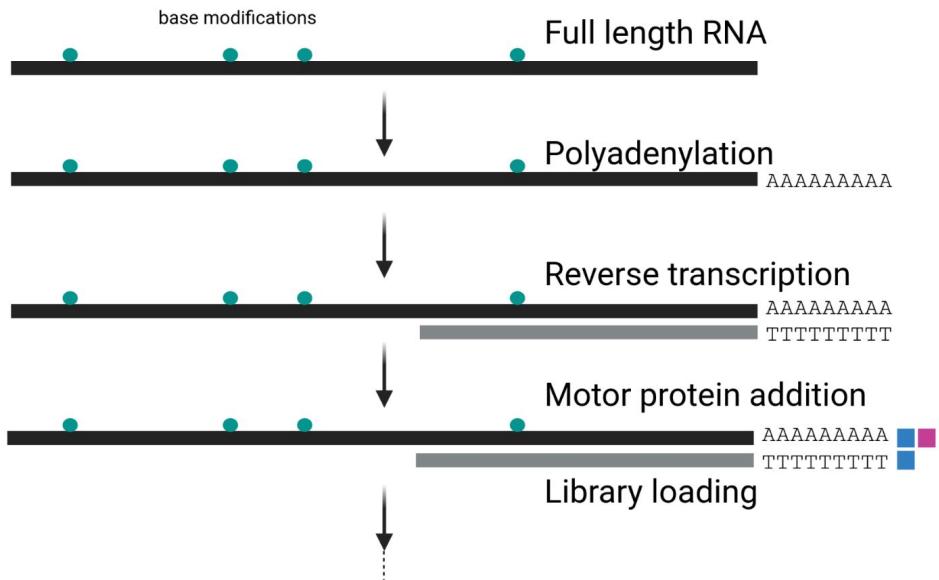
RNA-seq hints

- 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)

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

1 : ATGGTGTGCTGGTGCCTCTGGATGAGATCAGACAGGTCAGAGAGCTGA : 56 4488762 : ATGGTGTGCTGGTGCCTCTGGATGAGATCAGACAGGTCAGAGAGCTGA : 4488817 57 : TGGACCTGCAGGCATCTGGCTATTGGCACTGCTAACCTGAGAACCATGTGCTTC : 112 4488818 : TGGACCTGCAGGCATCTGGCTATTGGCACTGCTAACCTGAGAACCATGTGCTTC : 4488873 113 : AGGGGGAGTACTCTGACTACTCTCCGCATACCAACAGTGAACACATGACCAC : 168 4488874 : AGGGGGAGTACTCTGACTACTCTCCGCATACCAACAGTGAACACATGACCAC : 4488929 169 : CTCAAGGAGAAAGTTCAAGCGCATGT >>> Target Intron 1 >>> GC : 195 + + 86 bp + 4488930 : CTCAAGGAGAAAGTTCAAGCGCATGTgt.....agGC : 4489042 196 : GACAAGTCGACAATTGGAAACGTACATGCTACATCTGACGGAGGAATTCCCTCAAGGA : 251 4489043 : GACAAGTCGACAATTGGAAACGTACATGCTACATCTGACGGAGGAATTCCCTCAAGGA : 4489098 252 : AAACCCCACACATGTGCTTACATGGCTCTCTGGACACCCAGACAGGACATCG : 307 4489099 : AAACCCCACACATGTGCTTACATGGCTCTCTGGACACCCAGACAGGACATCG : 4489154 308 : TGGTGGTCAAGTCCCTAACGCTAGGCAAAGAAGCGGCAGTGAAGGCCATCAAGGAG : 363 4489155 : TGGTGGTCAAGTCCCTAACGCTAGGCAAAGAAGCGGCAGTGAAGGCCATCAAGGAG : 4489210	1 : MetValMetAlaGlyAlaSerSerLeuAspGluIleArgGlnAlaGlnArgAlaAs : 19 4488762 : MetValMetAlaGlyAlaSerSerLeuAspGluIleArgGlnAlaGlnArgAlaAs : 4488816 20 : pGlyProAlaGlyIleLeuAlaIleGlyThrAlaAsnProGluAsnHisValLeuG : 38 4488817 : pGlyProAlaGlyIleLeuAlaIleGlyThrAlaAsnProGluAsnHisValLeuG : 4488873 39 : lnAlaGluTyrProAspTyrPheArgIleThrAsnSerGluHisMetThrAsp : 56 4488874 : lnAlaGluTyrProAspTyrPheArgIleThrAsnSerGluHisMetThrAsp : 4488927 57 : LeuLysGluLysPheLysArgMet(C) >>> Target Intron 1 >>> : 65 + + 86 bp + 4488928 : LeuLysGluLysPheLysArgMet(C)++...ag : 4489039 66 : {ys}AspLysSerThrIleArgLysArgHisMetHisLeuThrGluGluPheLeuL : 83 { } { }{ }{ } 4489040 : {ys}AspLysSerThrIleArgLysArgHisMetHisLeuThrGluGluPheLeuL : 4489094 84 : ysGluAsnProHisMetCysAlaTyrMetAlaProSerLeuAspThrArgGlnAsp : 101 4489095 : ysGluAsnProHisMetCysAlaTyrMetAlaProSerLeuAspThrArgGlnAsp : 4489148 102 : IleValValValGluValProLysLeuGlyLysGluAlaIleValLysAlaIleLy : 120 4489149 : IleValValValGluValProLysLeuGlyLysGluAlaIleValLysAlaIleLy : 4489205 ATCGTGGTGTGAAAGTCCCTAACGCTAGGCAAAGAACGGCAGTGAAGGCCATCAA : 4489205
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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
- NL Rome: 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

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/>)

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/SOOT>
 - 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

Basic Local Alignment Search Tool

BLAST finds regions of similarity between biological sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance. [Learn more](#)

NEWS

BLAST+ 2.13.0 is here!
Starting with this release, we are including the blastn_vdb and tblasn_vdb executables in the BLAST+ distribution.

Thu, 17 Mar 2022 12:00:00 EST [More BLAST news...](#)

Web BLAST

Nucleotide BLAST
Nucleotide ▶ nucleotide

blastx
translated nucleotide ▶ protein

tblastn
protein ▶ translated nucleotide

Protein BLAST
protein ▶ protein

BLAST Genomes

Enter organism common name, scientific name, or tax id **Search**

Human Mouse Rat Microbes

Standalone and API BLAST

[Download BLAST](#)
Get BLAST databases and executables

[Use BLAST API](#)
Call BLAST from your application

[Use BLAST in the cloud](#)
Start an instance at a cloud provider

Specialized searches

SmartBLAST
Find proteins highly similar to your query

Primer-BLAST
Design primers specific to your PCR template

Global Align
Compare two sequences across their entire span (Needleman-Wunsch)

CD-search
Find conserved domains in your sequence

IgBLAST
Search immunoglobulins and T cell receptor sequences

VecScreen
Search sequences for vector contamination

CDART
Find sequences with similar conserved domain architecture

Multiple Alignment
Align sequences using domain and protein constraints

MOLE-BLAST
Establish taxonomy for uncultured or environmental sequences



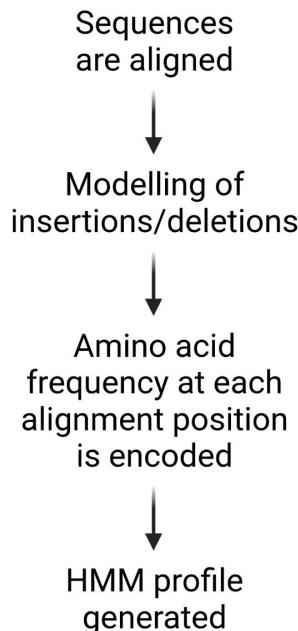
Pfam: Protein family database

HOME | SEARCH | BROWSE | FTP | HELP | ABOUT

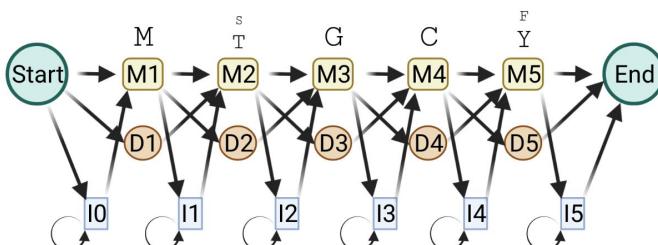
Pfam 35.0 (November 2021, 19632 entries)

The Pfam database is a large collection of protein families, each represented by **multiple sequence alignments** and **hidden Markov models (HMMs)**. [More...](#)

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



seq1	MTGC - Y	2 = deletion
seq2	MSGC - F	5 = insertion
seq3	MTGC - Y	
seq4	M - GCAY	
	1 2 3 4 5 6	



QUICK LINKS YOU CAN FIND DATA IN PFAM IN VARIOUS WAYS...

- [SEQUENCE SEARCH](#) Analyze your protein sequence for Pfam matches
- [VIEW A PFAM ENTRY](#) View Pfam annotation and alignments
- [VIEW A CLAN](#) See groups of related entries
- [VIEW A SEQUENCE](#) Look at the domain organisation of a protein sequence
- [VIEW A STRUCTURE](#) Find the domains on a PDB structure
- [KEYWORD SEARCH](#) Query Pfam by keywords

[JUMP TO](#) [Go](#) [Example](#)

Enter any type of accession or ID to jump to the page for a Pfam entry or clan, UniProt sequence, PDB structure, etc.

Or view the [help](#) pages for more information

Recent Pfam blog posts

[Pfam 35.0 Is released](#) (posted 19 November 2021)

Pfam 35.0 contains a total of 19,632 families and clans. Since the last release, we have built 460 new families, killed 7 families and created 12 new clans. UniProt Reference Proteomes has increased by 7% since Pfam 34.0, and now contains 61 million sequences. Of the sequences that are in UniProt Reference Proteomes, 75.2% have [...]

[AlphaFolding the Protein Universe](#) (posted 22 July 2021)

Hot on the tail of our inclusion of the Baker group's trRosetta structural models we are excited to announce the inclusion of models from AlphaFold 2.0 generated by DeepMind and stored in the AlphaFold Database (AlphaFold DB). AlphaFold 2.0's performance in the CASP14 competition was spectacular, producing near experimental quality structure models. The new AlphaFold [...]

[Google Research Team bring Deep Learning to Pfam](#) (posted 24 March 2021)

We are delighted to announce the first fruits of a collaboration between the Pfam team and a Google Research team led by Dr Lucy Colwell, with Maxwell Bileschi and David Belanger. In 2019, Colwell's team published a preprint describing a new deep learning method that was trained on Pfam data, and which improves upon the [...]

Citing Pfam

If you find Pfam useful, please consider [citing](#) the reference that describes this work:

[Pfam: The protein families database in 2021](#): J. Mistry, S. Chuguransky, L. Williams, M. Qureshi, G.A. Salazar, E.L.L. Sonnhammer, S.C.E. Tosatto, L. Paladini, S. Raj, L.J. Richardson, R.D. Finn, A. Bateman

[Nucleic Acids Research](#) (2020) doi: 10.1093/nar/gkaa913

Pfam is part of the ELIXIR infrastructure
Pfam is an ELIXIR service [Read more](#)

Comments or questions on the site? Send a mail to pfam-help@ebi.ac.uk
European Molecular Biology Laboratory

<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

InterProScan 5 Sequence Search

This form allows you to scan your sequence for matches against the InterPro collection of protein signature databases. This form is for debugging purposes only and is **not supported**. To submit jobs to InterProScan 5, please visit the [InterPro Sequence Search](#) or the [InterProScan 5 Web services](#).

Please Note

This web form is for internal debugging purposes and will be retired in April 2016. If you have any questions/concerns please contact us via the feedback link above.

STEP 1 - Enter your input sequence

Enter or paste a PROTEIN sequence in any supported format:
uniprot:KPYMF_HUMAN

Or, upload a file Choose File | No file chosen Use a example sequence | Clear sequence | See more example inputs

STEP 2 - Select the applications to run

TIGRFAM SFLD Phobius SignalP SignalP_EUK
 SignalP_GRAM_POSITIVE SignalP_GRAM_NEGATIVE SUPERFAMILY PANTHER Gene3D
 Hmmp ProSiteProfiles ProSitePatterns Coils SMART
 CDD PRINTS Pfam MobidBlite
 TMHMM

STEP 3 - Submit your job

Be notified by email (Tick this box if you want to be notified by email when the results are available)

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

Tool Output Submission Details

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:KPYMF_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 alignment 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
```

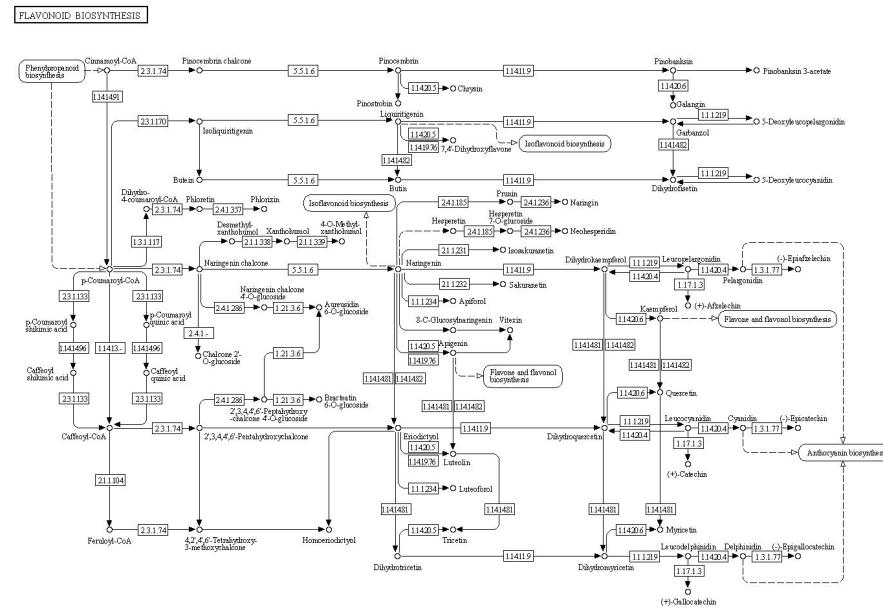
where INPUT_FASTA is a fasta file containing the amino acid sequence for the search and SHOOT_DB is the SHOOT database directory created using the steps above.

<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)

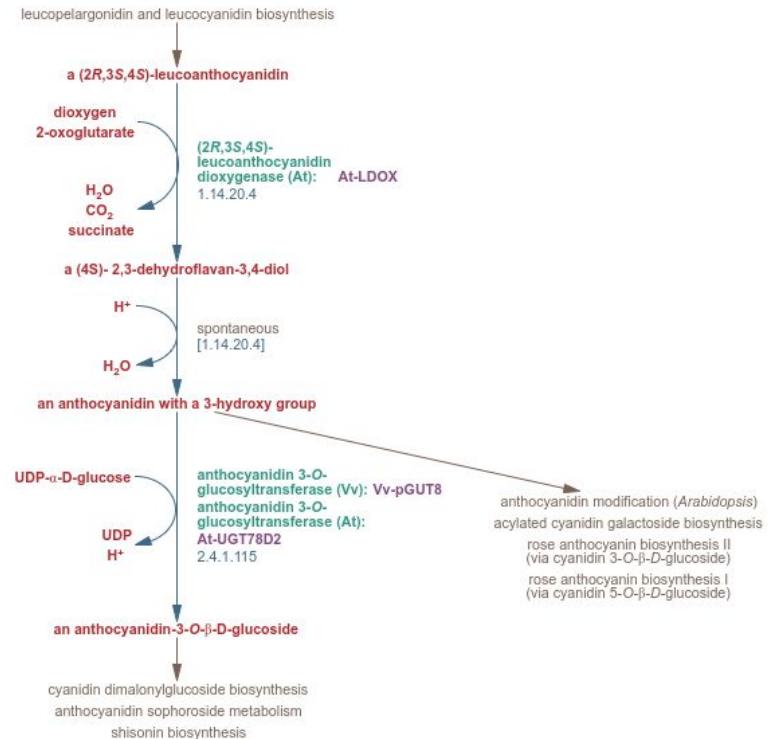
- Defined statements about the function of a gene (controlled vocabulary)
- Hierarchical structure
 - Example: ‘metabolic process’ > ‘biosynthetic process’ > ... > ‘chalcone synthase’
- Supported by the Alliance of Genome Resources
- Connected with various other databases e.g. TAIR, FlyBase, Reactome,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

■ GO:0008150 biological_process
■ GO:0008152 metabolic process
■ GO:0009058 biosynthetic process
■ GO:0071704 organic substance metabolic process
■ GO:0009812 flavonoid metabolic process
■ GO:1901576 organic substance biosynthetic process
▼ GO:0009813 flavonoid biosynthetic process
■ GO:0009718 anthocyanin-containing compound biosynthetic process
■ GO:0051551 aurone biosynthetic process
■ GO:0033485 cyanidin 3-O-glucoside biosynthetic process
■ GO:0033486 delphinidin 3-O-glucoside biosynthetic process
■ GO:0051553 flavone biosynthetic process
■ GO:0009716 flavonoid phytoalexin biosynthetic process
■ GO:0051557 leucoanthocyanidin biosynthetic process
■ GO:0009964 negative regulation of flavonoid biosynthetic process
■ GO:0033487 pelargonidin 3-O-glucoside biosynthetic process
■ GO:0009963 positive regulation of flavonoid biosynthetic process
■ GO:0009962 regulation of flavonoid biosynthetic process

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

MetaCyc: Metabolite Encyclopedia

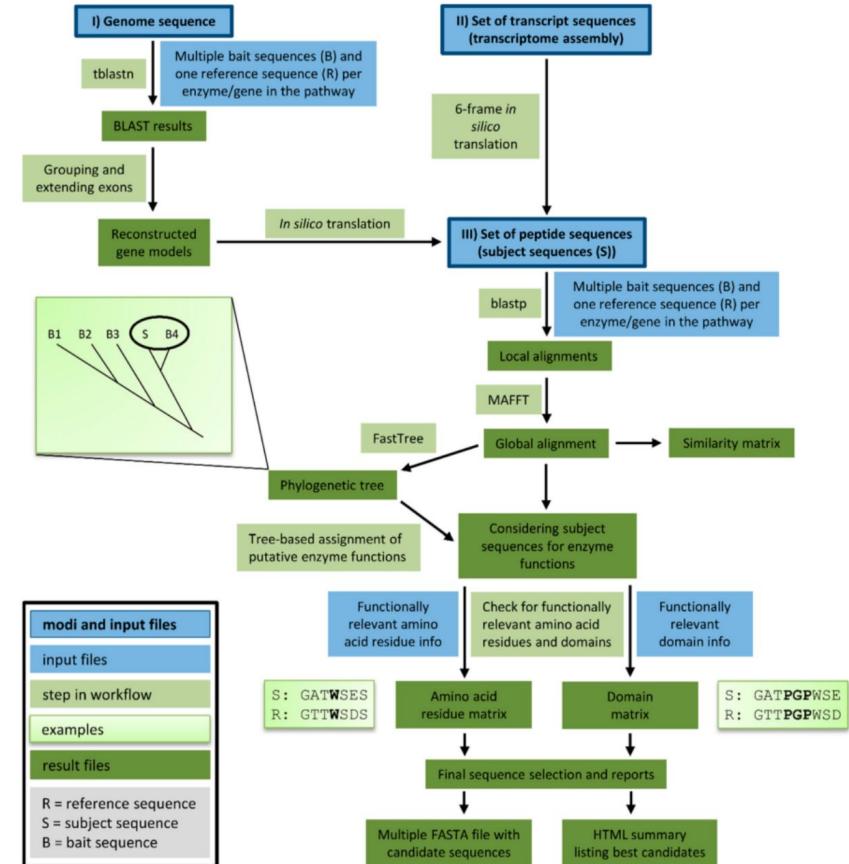
- Integrates genomic data with functional annotation
- Visualization of pathway databases
- Shows intermediates and enzymes of biosynthesis pathways
- MetaFlux: flux-balance analysis



<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>

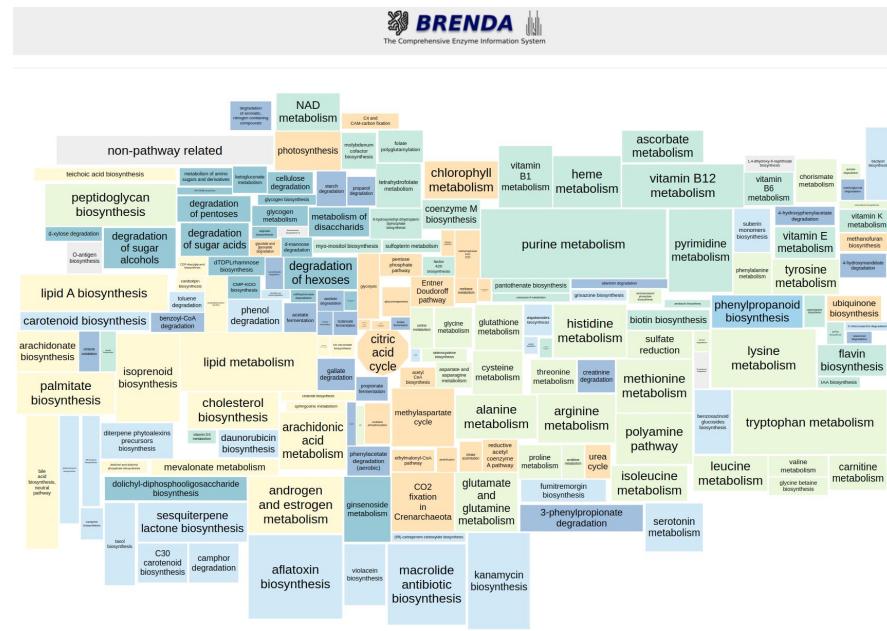
KIPeS

- 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/>



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
MIGPMPGMEGKLMPLPGASPVGLEVVLVASTPILSDTSLCTPSFYHFLLLGPITSISNLIVRPFLLSSITVIYGRLCFFAFDFYVY
>TRINITY_DN100016_c0_g1_i1
MPKKSSNIQQNNQNRRIKRKGRRPKKKYVQQIDSSDEDILSVRHASTRPRIISIRRNEIPMRPEIHIPPTSIPSFNNPNNIHQSSSDRQMPP
>TRINITY_DN100019_c0_g1_i1
MPPKAADKKPAAKAPATASKAPEKKDAGKKTAAATGDKKRTKARKETYSSYIYKVLKQVHPDTGISNRAMSILNSFVNDFERVATEASKLA
>TRINITY_DN10001_c0_g1_i2
MAKVGNPVIDETDGSVNEPESSEKNIEVSSSTQAPESTNTTELLVNEKKAFSLATPAVRRVAREHNIDINNIKGTGKNGRITKEDILNYV
>TRINITY_DN100025_c0_g1_i1
MVENQDGCFKPGWKEFVRNSDLEGGDFLVNLVDKISYQVVIFDGTCACPDKLCPFSIMNPIFIQHLRNKIFLSKKEEIKLKGNRKVHSVNEN
>TRINITY_DN10002_c0_g1_i1
MSDELNQVFQRCREQKRPVVFVAFVTAGYPDSEETVDILLGLEAGGADIIIELGIPFTDPMVDGKTIQDANNVALENKIDISKCLSYVSESRAK
```

<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?