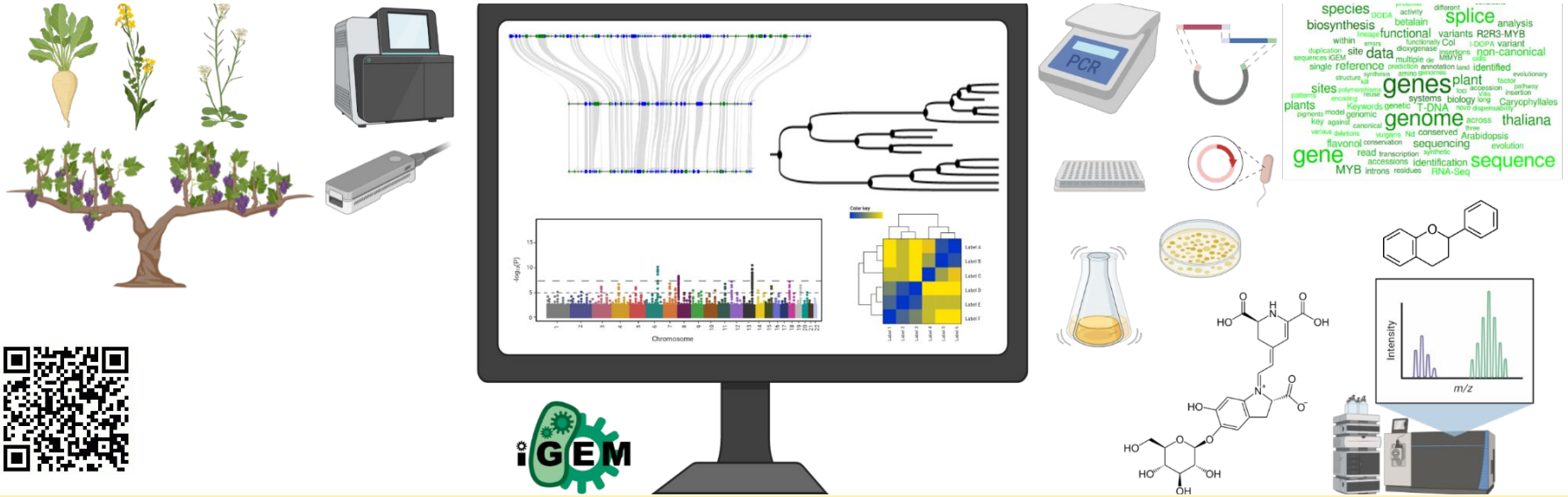
Technische Universität Braunschweig



Genome Sequence Annotation

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

Availability of slides

- All materials are freely available (CC BY) - after the lectures:
  - StudIP: **GE31/MM12**
  - GitHub: https://github.com/bpucker/teaching

- Questions: Feel free to ask at any time

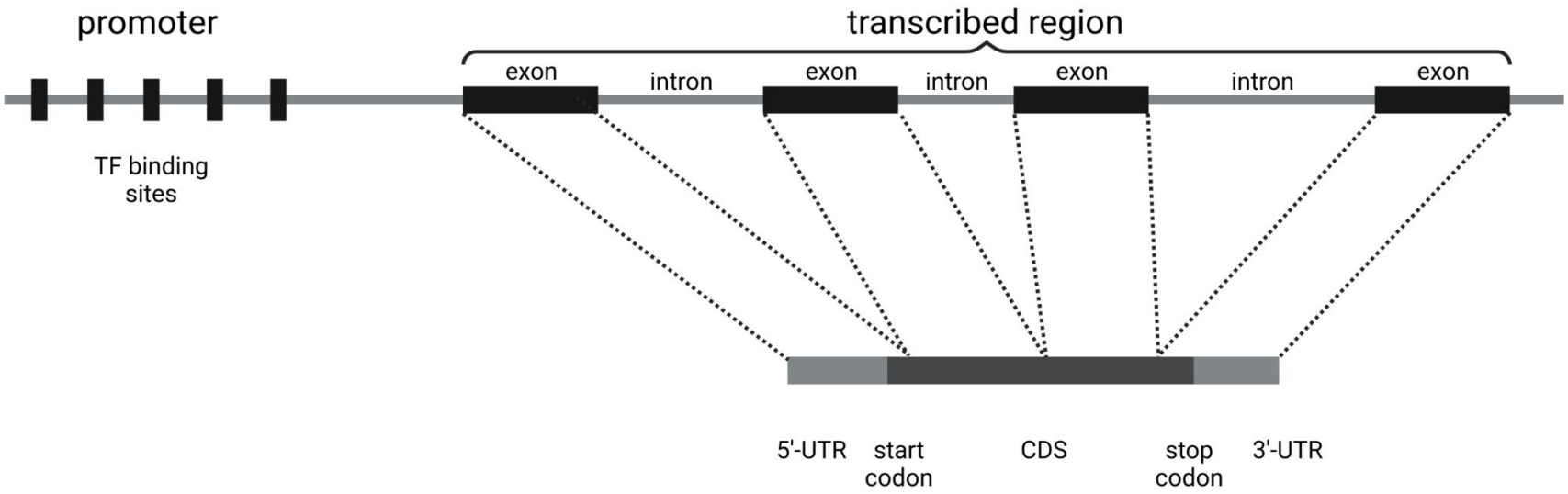
- Feedback, comments, or questions: b.pucker[a]tu-braunschweig.de

Finding genes in a genome sequence

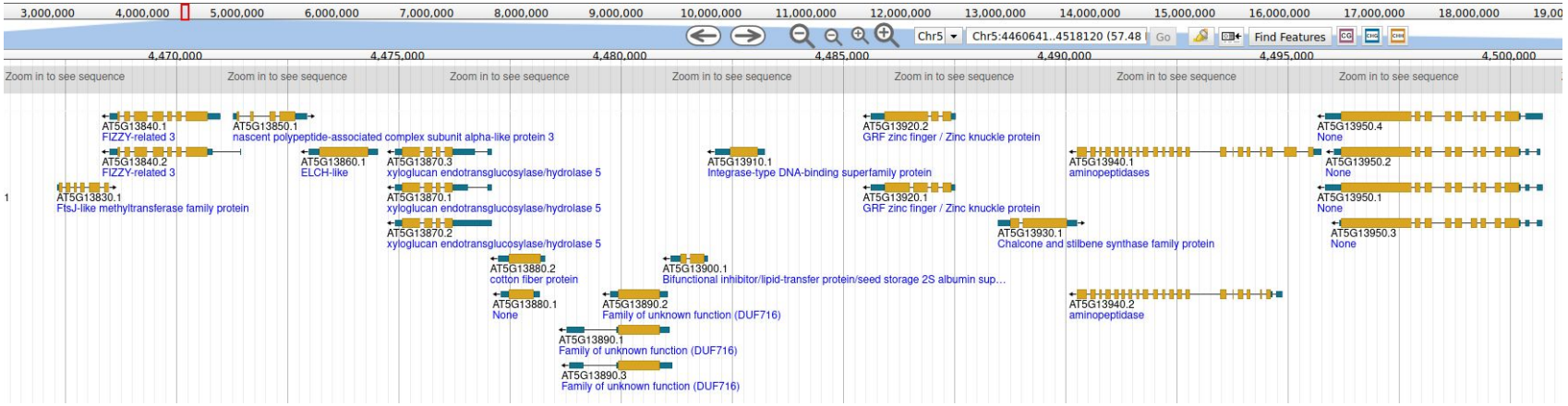
Siadjeu & Pucker et al., 2020: 10.3390/genes11030274

Plant gene structure

CDS = (Protein) Coding Sequence
ORF = Open Reading Frame

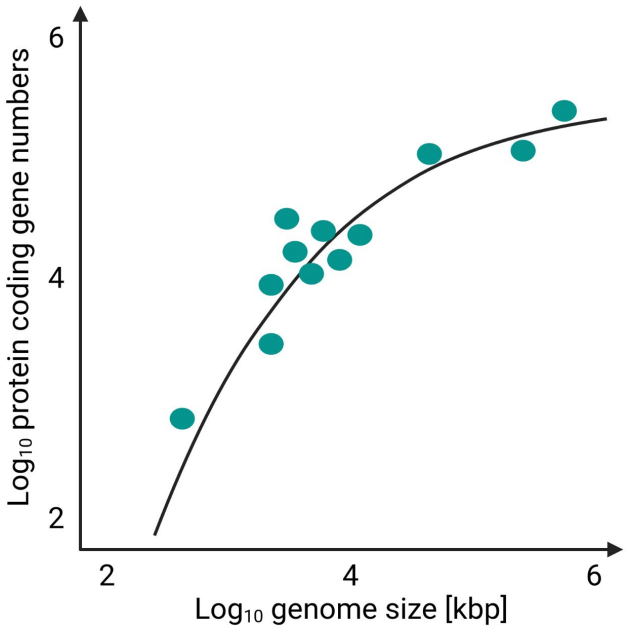


Finding genes in a genome sequence



Gene numbers

- Average number of genes in plants: 27200
- Gene number is not significantly correlated with genome size

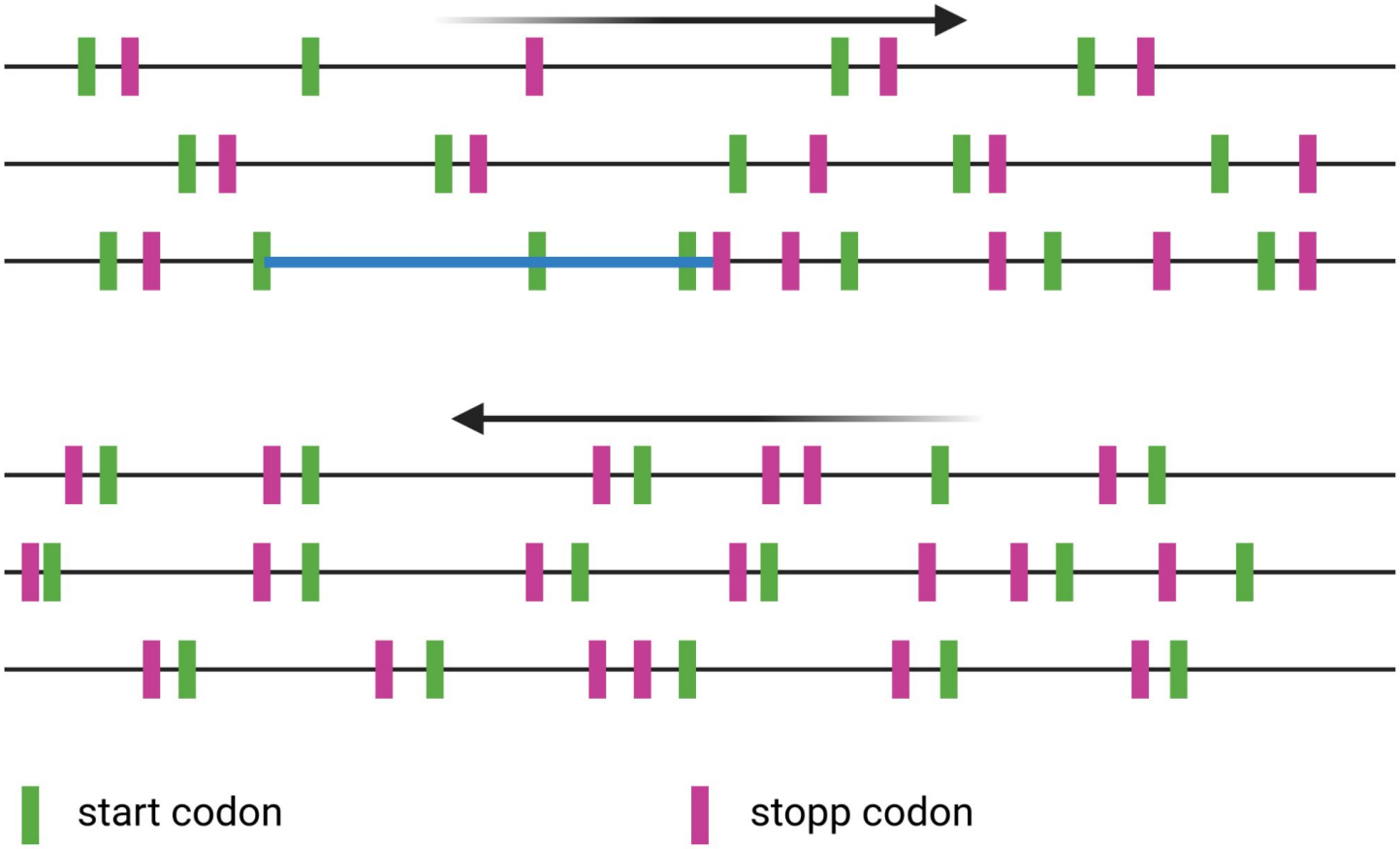


Pucker & Brockington, 2019: 10.1186/s12864-018-5360-z
Michael, 2014: 10.1093/bfgp/elu005

Repeat masking

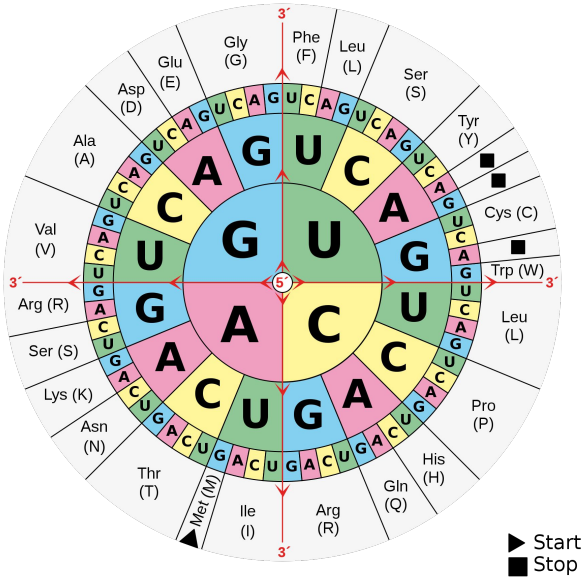
- Simple sequence repeats (SSR)
- Transposable elements (TE)
- Centromeric repeats (CEN)
- Telomeric repeats (TEL)

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



How does a Hidden Markov Model work?

Hidden Markov Models (1)

Result	Fair	Loaded
1	1/6	1/10
2	1/6	1/10
3	1/6	1/10
4	1/6	1/10
5	1/6	1/10
6	1/6	1/2

Hidden Markov Models (2)

- Observation: 1-4-6-2-4-3-2-6-1-5
- Probability using fair die (F):
  - $(1/6)^{10} = 1.6538 * 10^{-8}$
- Probability using loaded die (L):
  - $^1/_{10} * ^1/_{10} * ½ * ^1/_{10} * ^1/_{10} * ^1/_{10} * ^1/_{10} * ½ * ^1/_{10} * ^1/_{10} = 2.7778 * 10^{-10}$

Hidden Markov Models (3)

- Observation:

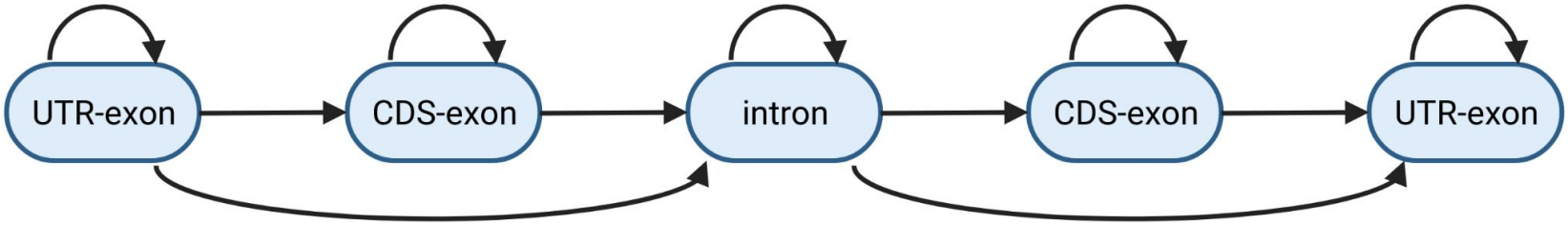
  1625453666166152316342423315266261662656462624351354

  FFFFFFFLLLLLLFFFFFFFFFFFFFFFLLLLLLLLLLLLLLLLFFFFFFFF

- How does this fit to gene prediction?

Hidden Markov Model for gene prediction

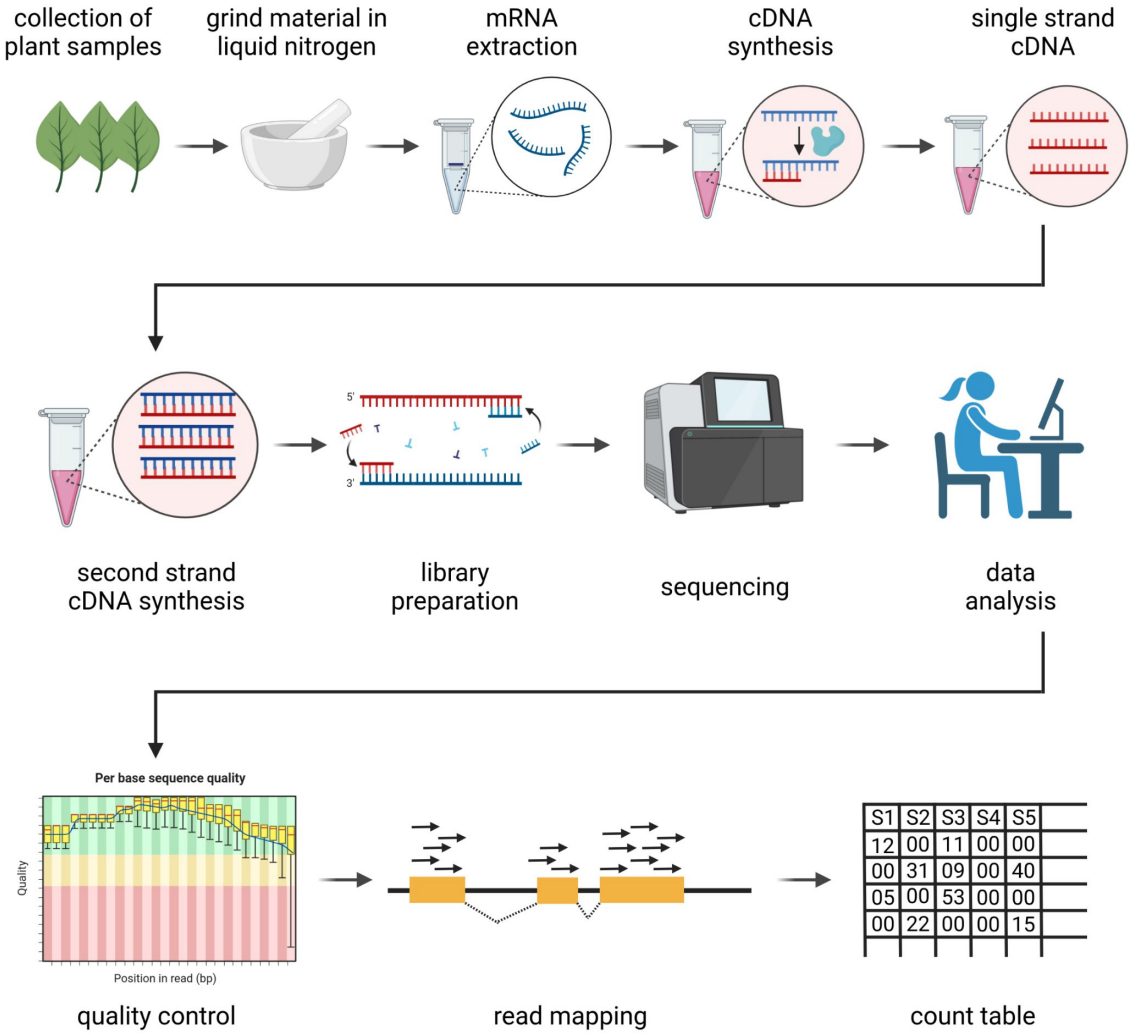
- Fair die = exon (GC-rich in plants)
- Loaded die = intron (AT-rich in plants)
- Switch between fair die and loaded die at intron/exon borders



Parameters for *ab initio* gene prediction

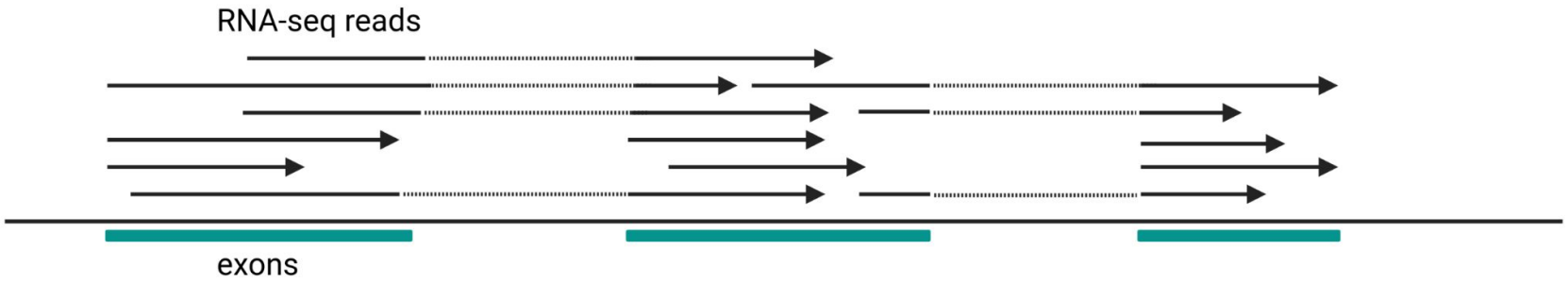
- Features: utr, exon, intron, intergenic region
- Composition of different features (dicodons)
- feature lengths
- Number of features per gene
- Possible splice sites

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

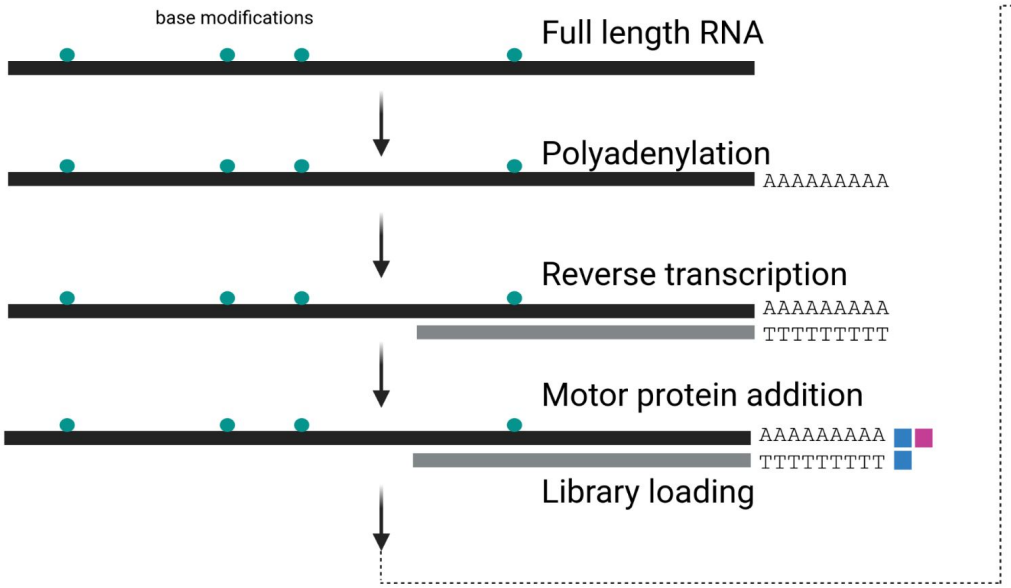
RNA-seq hints

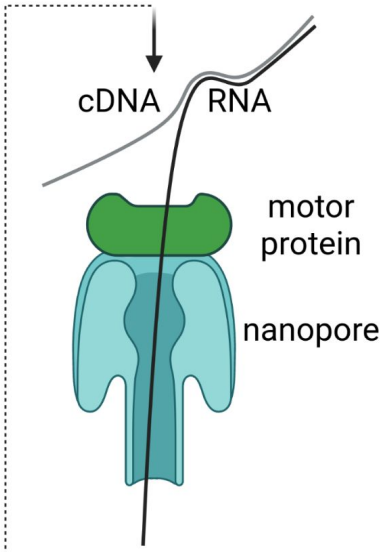
- Aligned RNA-seq reads indicate exon positions
- Splitting of reads indicates intron positions
- CDS can be identified as ORF within the covered regions



Full length transcript sequences

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



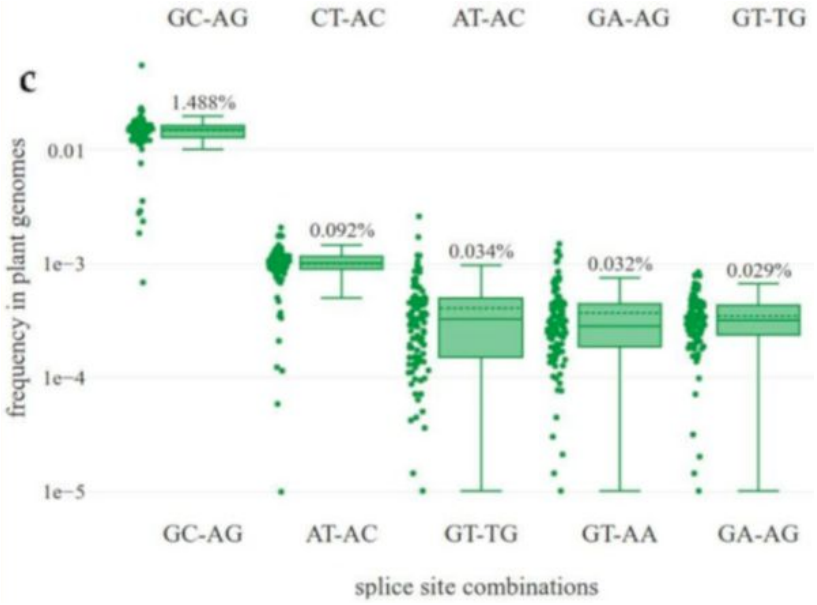


Canonical splice sites

- Spliceosome catalyzes the removal of introns from pre-mRNA
- Recognition of intron/exon borders required
- Splice sites are recognition sites at the intron borders

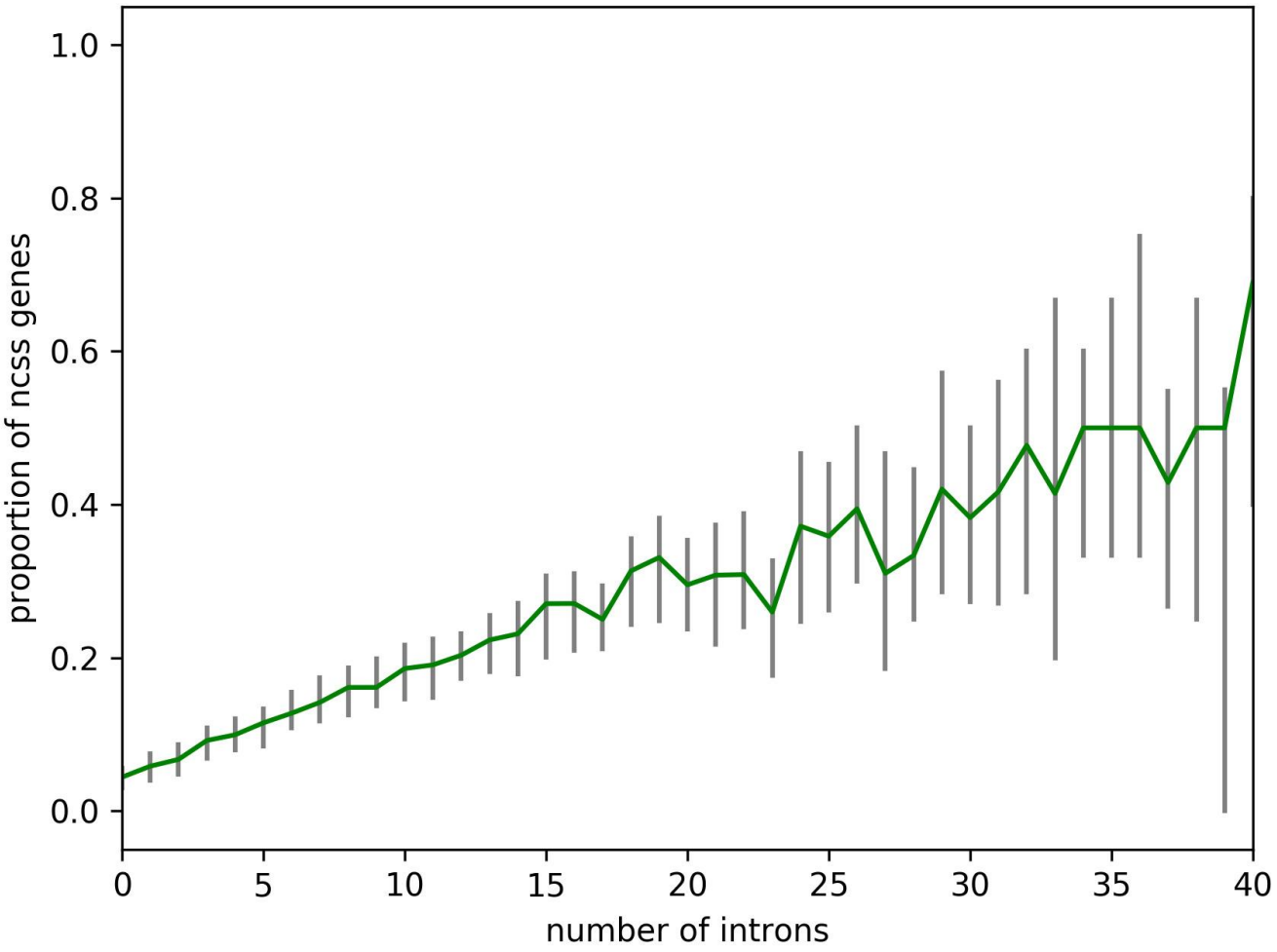
Non-canonical splice sites

- Splice sites mark points for removal of introns
- Highly conserved to allow recognition by spliceosome
- Two types of spliceosomes: U2 and U12
- Canonical splice sites (98%): GT-AG
- Major non-canonical splice sites (1-2%): GC-AG, AT-AC
- Minor non-canonical splice sites (<1%): NN-NN



Frey & Pucker, 2020: 10.3390/cells9020458

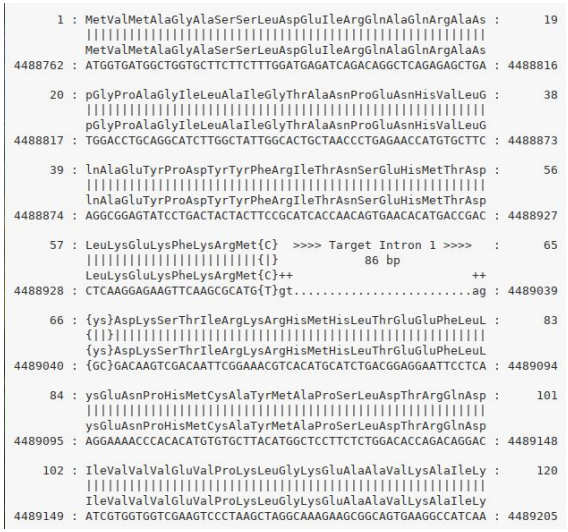
Relevance of non-canonical splice sites



Pucker & Brockington, 2018: 10.1186/s12864-018-5360-z

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

Identification of tRNAs

- tRNA genes have RNA gene products
- tRNAscan-SE2 is a dedicated tool for the annotation of tRNAs
- GtRNAdb contains the results of tRNAscan-SE (http://gtrnadb.ucsc.edu/)

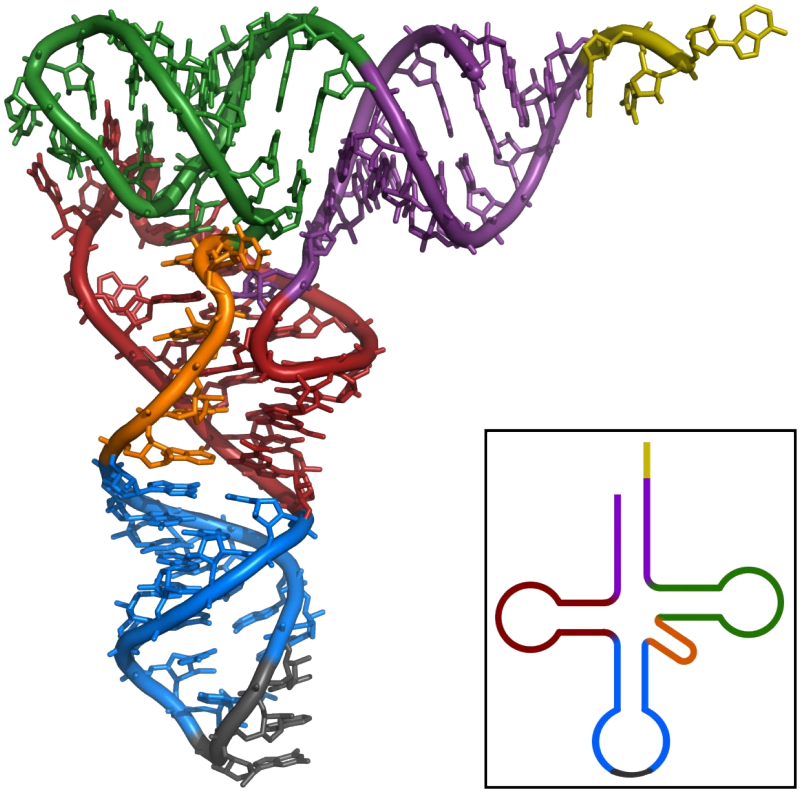
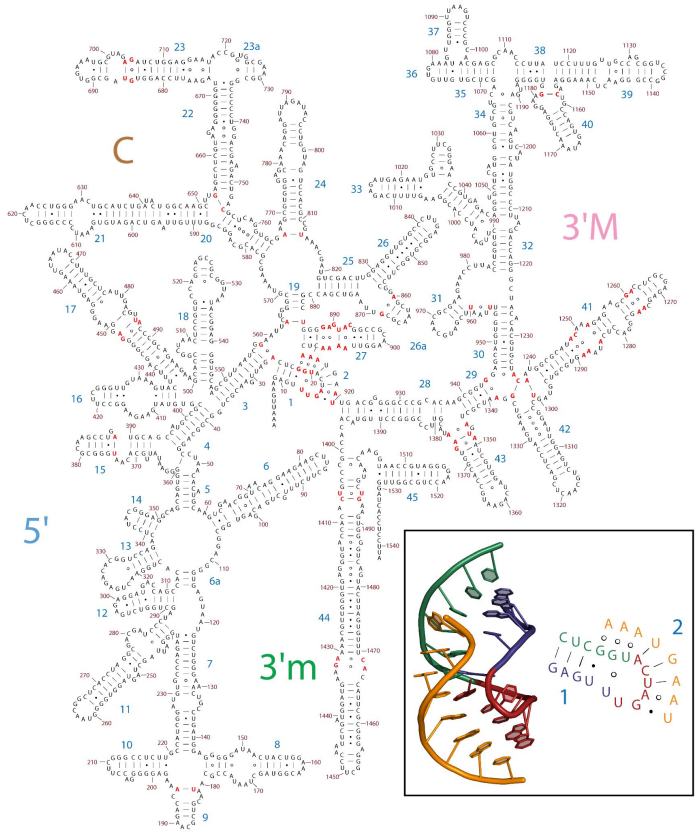


Figure source: https://commons.wikimedia.org/wiki/File:TRNA-Phe_yeast_1ehz.png

Chan & Lowe, 2019: 10.1093/nar/gkab688

Identification of rRNAs

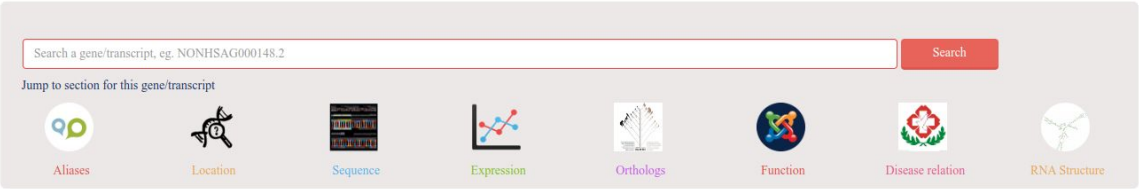
- Rfam: database of RNA genes (http://rfam.xfam.org/)
- RepeatMasker can identify rRNAs
- RNAmmer is dedicated tool for annotation of rRNAs



Kalvari et al. , 2017: 10.1093/nar/gkx1038
Lagesen et al., 2007: 10.1093/nar/gkm160
Petrov et al., 2014: 10.1371/journal.pone.0088222

Annotation of long non-coding RNAs (lncRNAs)

- NONCODEV6: long non-coding RNA database (http://www.noncode.org/)
- Identification based on sequence similarity



Zhao et al., 2020: 10.1093/nar/gkaa1046
http://www.noncode.org/

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

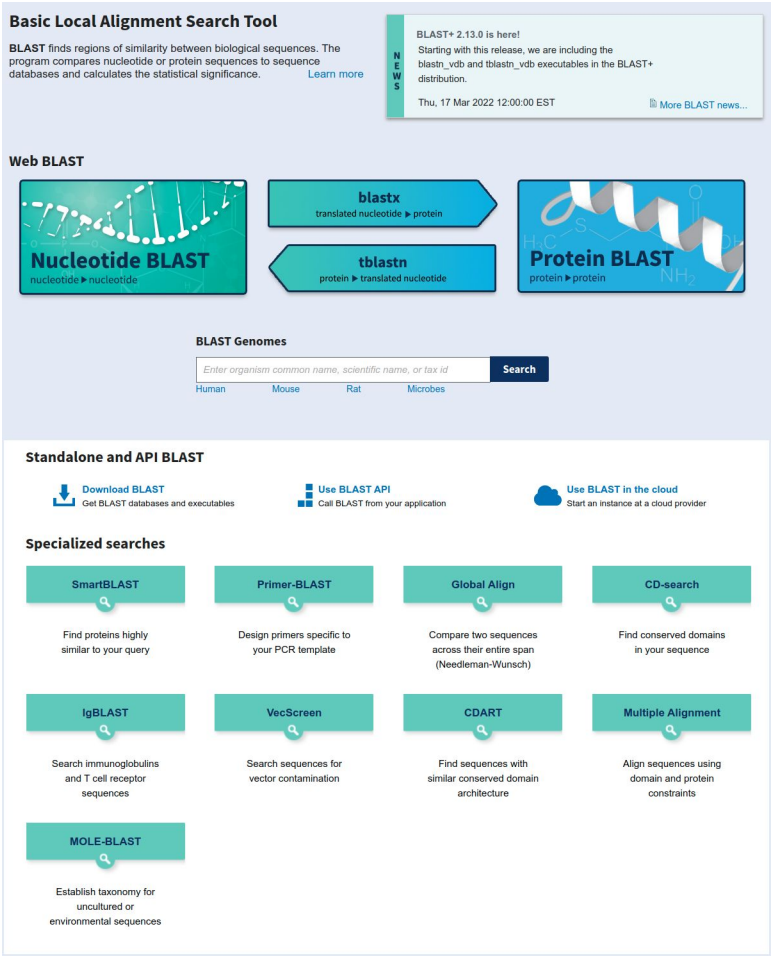
Functional annotation

- 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

↓

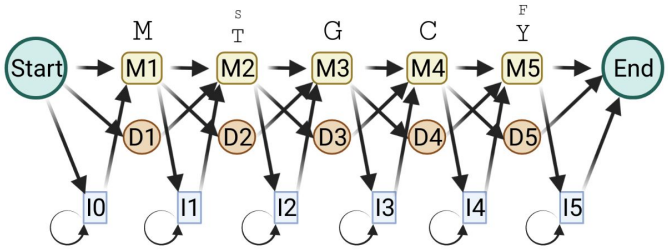
Amino acid frequency at each alignment position is encoded

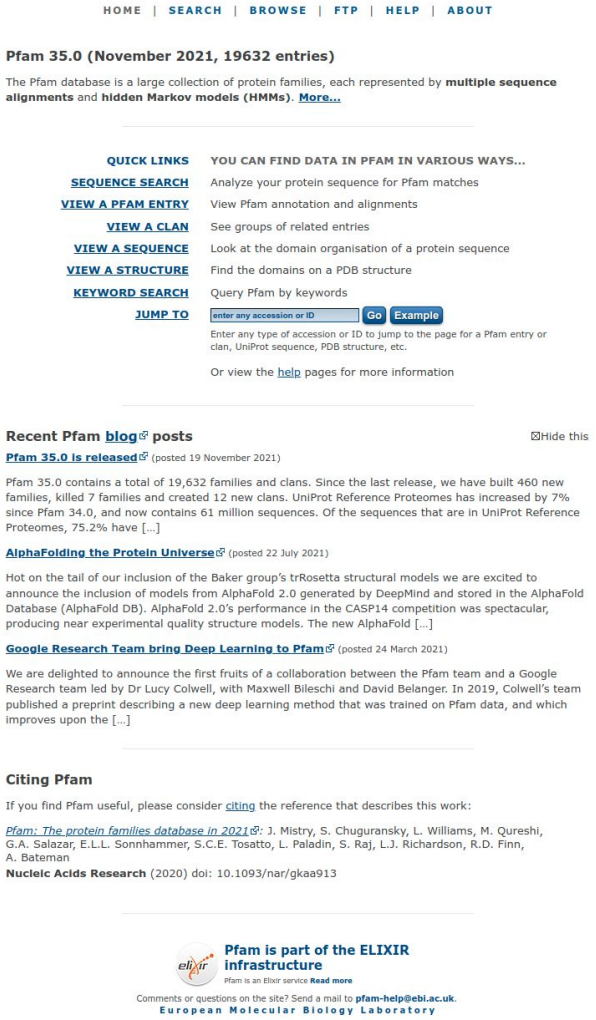
↓

HMM profile generated

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

2 = deletion
5 = insertion

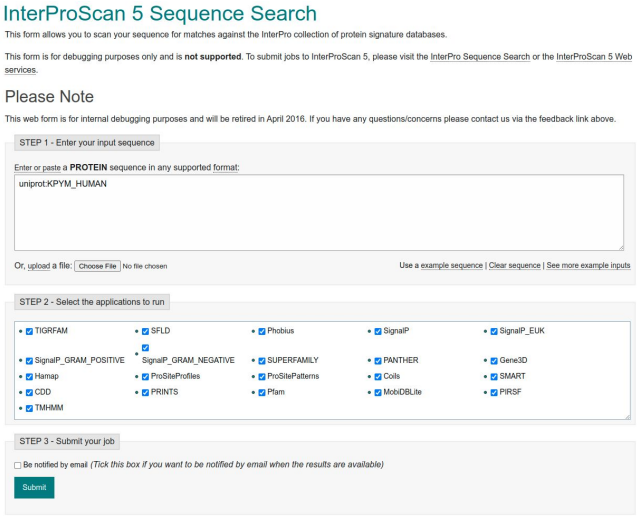




http://pfam.xfam.org/
Mistry et al., 2020: 10.1093/nar/gkaa913

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

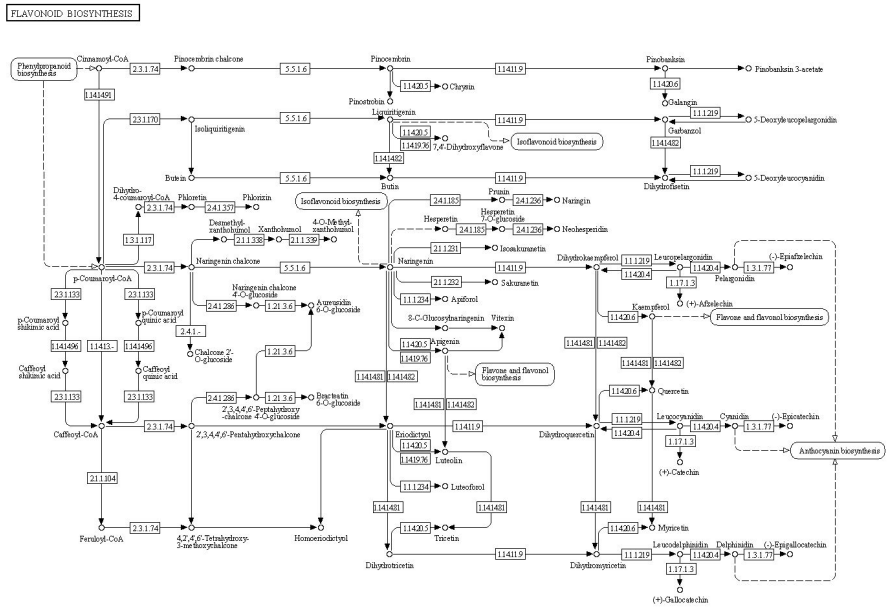
```
python shoot INPUT_FASTA SHOOT_DB
```

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

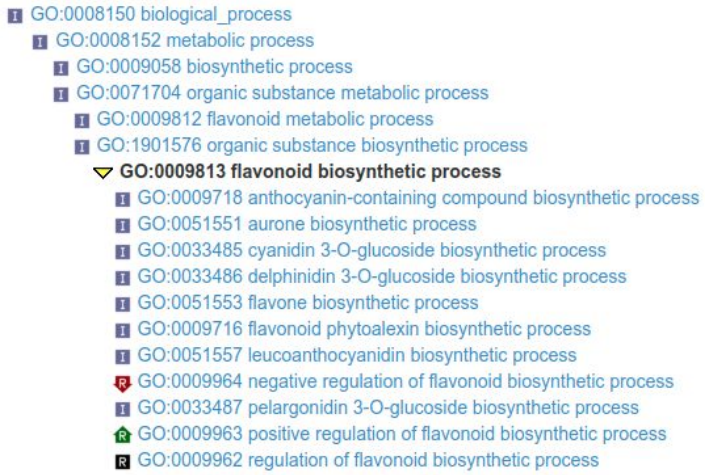


map00941 Flavonoid biosynthesis

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)

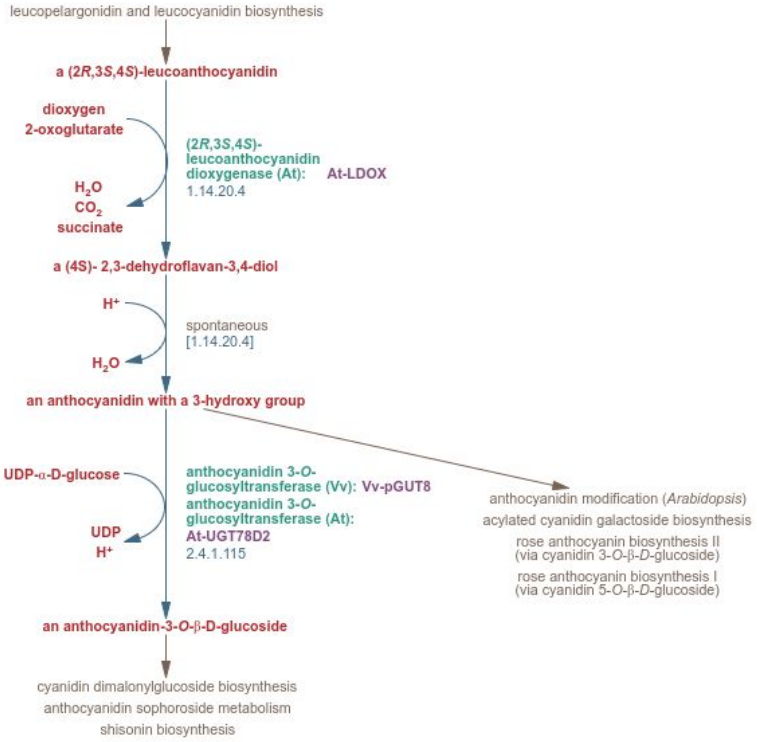
- Defined statements about the function of a gene (controlled vocabulary)
- Hierarchical structure
  - Example: ‘metabolic process’ > ‘biosynthetic process’ > … > ‘chalcone synthase’
- Supported by the Alliance of Genome Resources
- Connected with various other databases e.g. TAIR, FlyBase, Reactome, 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

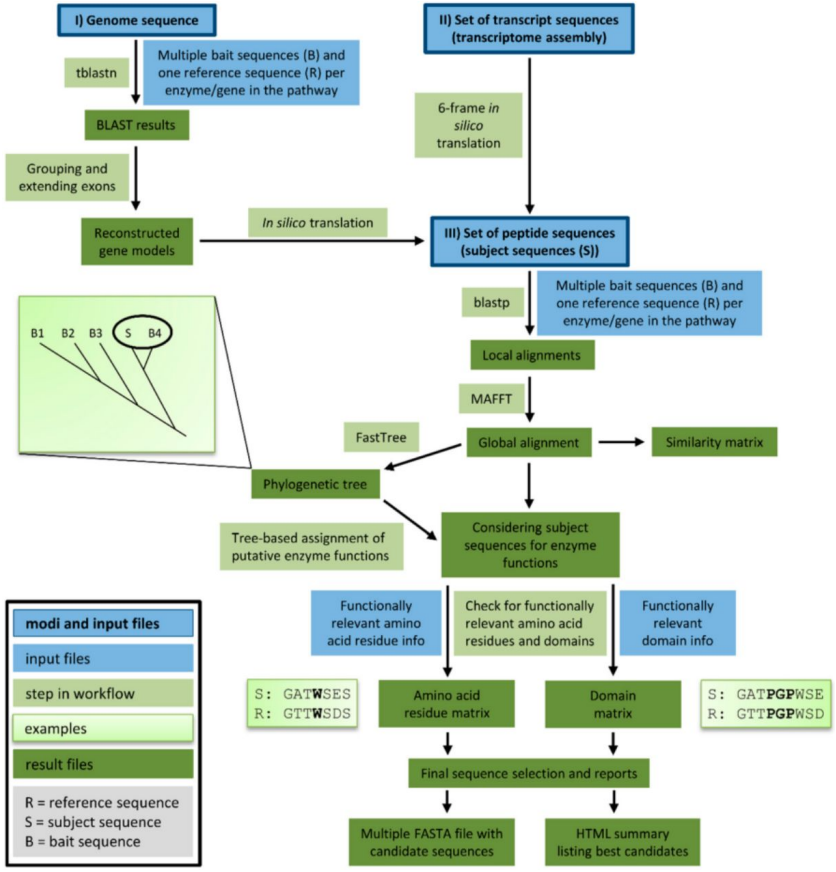
- Integrates genomic data with functional annotation
- Visualization of pathway databases
- Shows intermediates and enzymes of biosynthesis pathways
- MetaFlux: flux-balance analysis



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

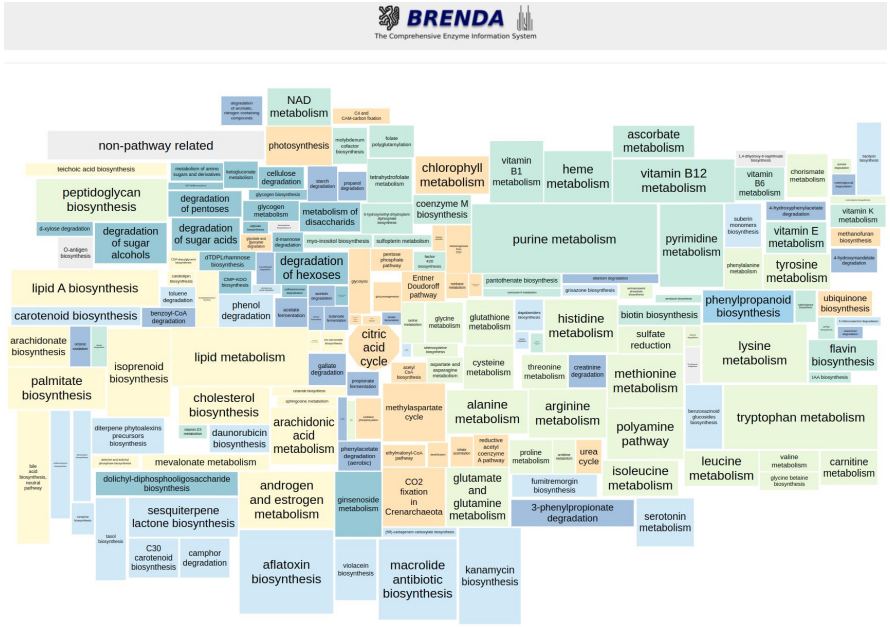
- Identification of all players in a biosynthesis pathway
- Dedicated to the identification of enzymes
- Functionally relevant amino acids need to be known
- Web server: http://pbb.bot.nat.tu-bs.de/KIPEs/



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

Time for questions!

Questions

1. What are the important features of a eukaroytic gene?
2. What are the important step of an RNA-seq experiment?
3. Which hints can be used in a gene prediction process?
4. How would you perform a gene prediction?
5. What is the relevance of non-canonical splice sites?
6. Which types of features can be annotated in a genome sequence?
7. Which tools are available for the annotation of different gene types?
8. How can TEs be annotated?
9. Which tools can be used for the functional annotation of genes?