Annotation of Protein Coding Genes

January 8th 2025

Katharina J. Hoff

Twitter: @katharina_hoff
Bluesky: @katharinahoff.bsky.social
Mastodon: @KatharinaHoff@fosstodon.org

E-Mail: katharina.hoff@uni-greifswald.de

Katharina J. Hoff
Group Leader in Applied Bioinformatics at University of Greifswald

Short CV

2005 B.Sc. Plant Biotechnology (Hanover, stays abroad: Budapest & Alnarp)

2009 Ph.D. Molecular Biology (Göttingen)

2022 Habilitation (Greifswald)

Research

- eukaryotic genome annotation, metagenomics
- best known for: **BRAKER** & other **Gaius-Augustus** software
- 37 peer-reviewed research articles with currently 7,186 citations

Teaching

- currently 1 postdoc, 4 PhD students, 1 MSc student, 2 BSc students
- applied bioinformatics, programming, statistics, & data science

... I love to sail, have a dog, a cat, and an 8-years old daughter...

After this lecture you will

- understand what genome annotation in eukaryotes is
- know the basics of a Hidden Markov Model
- have a vague idea of INSDC annotation pipelines
- roughly understand methods sections on genome annotation
- know what's happening in BRAKER & GALBA
- be aware of the rapid advances with Deep Learning
- have an idea of quality control methods

Materials at

`https://github.com/KatharinaHoff/GenomeAnnotation_Workshop`

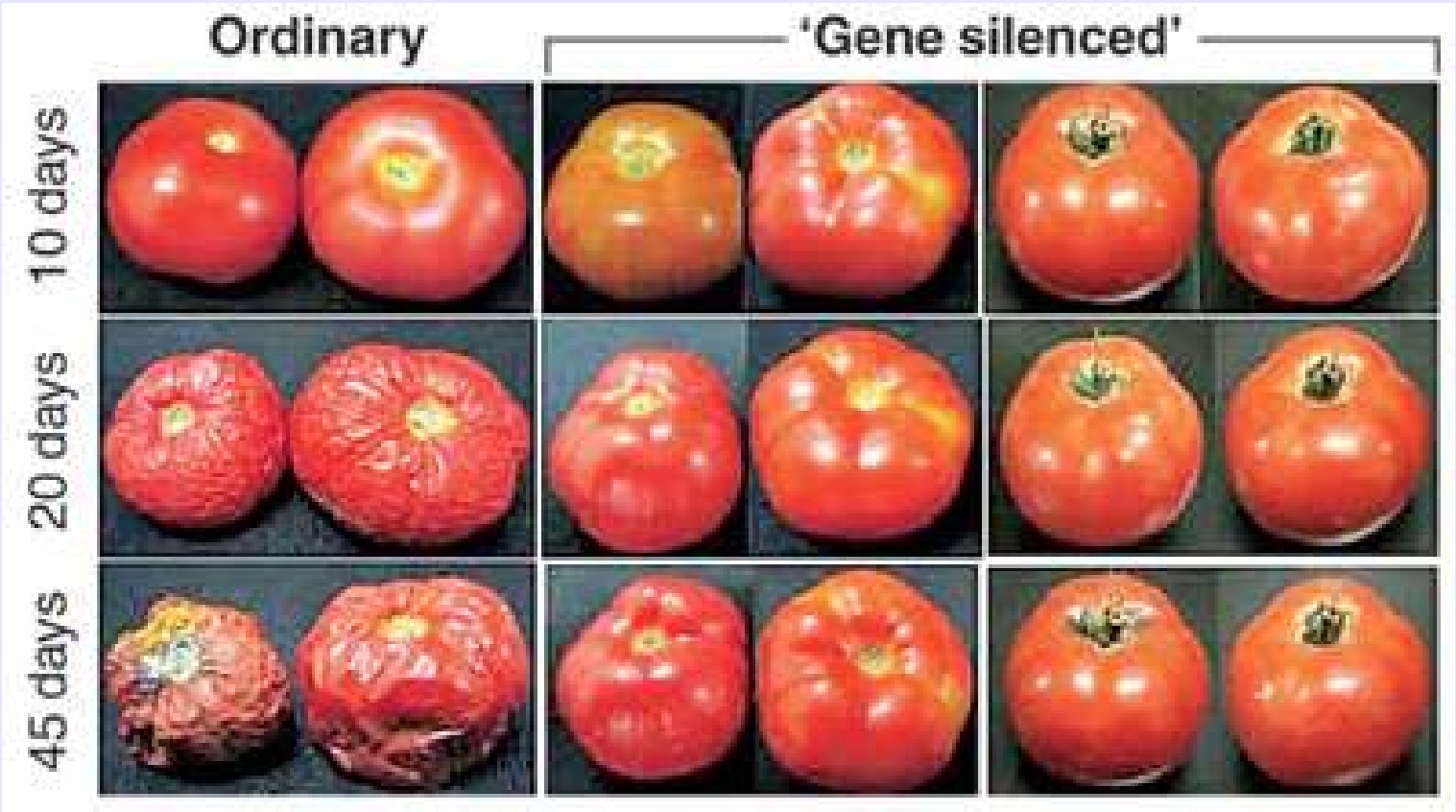
(Some images have been removed on Github because I do not have permission to share them.)

Where are the protein coding genes?

Genomic sequence: chicken

cctcacctctgagaaaacctctttgccaccaataccatgaagctctgcgtgactgtcctgtctctcctc
gtgctagtagctgccttctgctctctagcactctcagcaccaagtaagtctacttttgcagctgctatt
tcgagtcaaggtgtaggcagagtccttttttctagtcatggctggcaaacagtgggatctggggatggg
acaaaaggcagctaggaagattgccatgtagtctgctgctaaatgtagagtctagtagatattcagtaa
cattcaagttcctattttcttaagaattagcaaccagcagaggaaaacgatgggctggaagtcagactg
ttgaattggctctgcctttaattatttgttcaagcaagcccctgtccctctctgtgccttggtttcccc
atctgtcatatgaagggagtgcgatgtgttctgagactgaatccagttccaatcttctagatttctttc
tcgttcttctctgaagatccactattcagaataagactcctgctcatgttaggtgggaatggatacaag
ggaccatatttggggttctggtagctccacagggatgctcaatgaagatgcaaaattagaagtcaaaat
aaacagctcccatgggcagtgttgatctcaccctggcctttcctttcagtgggctcagaccctcccacc
gcctgctgcttttcttacaccgcgaggaagcttcctcgcaactttgtggtagattactatgagaccagc
agcctctgctcccagccagctgtggtgtgagtatcaacccctggctgccctgggaggcaagggtgaggg
ctggatttttaaaggggcctgttttggggaggggtgatgagcgctggggaggcagctctcagggctg
aagccttccctgacagcagtgaggtcacaggtcatgaactcacttttcaagtgctgaaggcggctgagt
ggcagccgagacagaaggggttcctggggaggaagttattcagaggacagggaagcaggggaaggcag
acaggtcccatgagatatggaccaattccttaaaccatgctagaaaaacatgtggaaaagtcactacca
ggctggcagggaatggggcaatctattcatactgattgcaatgcccactggttcctaatctgggcaacc
cctggggcccacagctaaatccagtgagtggaagttacagggagtctgcttccagtgctgctcgaggaa
ggatcccatccaccagagctgccccacatggaccatggtcaggcagaggaagatgcctaccacaggcaa
gggataaagccagatgacctcaaaggtcccatgggattctaatctgtctgctccttgttctacagattc
caaaccaaaagaggcaagcaagtctgcgctgaccccagtgagtcctgggtccaggagtacgtgtatgac
ctggaactgaactgagctgctcagagacaggaagtcttc

Examples for the importance of genome annotation



Sheeny et al. (1988) Proc. Natl. Acad. Sci. USA 85:8805-8809; Image: adapted from

`http://luisbarbosa2.blogspot.com/2013/06/flavr-savr-tomato.html`, Original: Asia Datta, Subhra Chakraborty, National Institute of Plant Genome Research, New Delhi

Examples for the importance of genome annotation

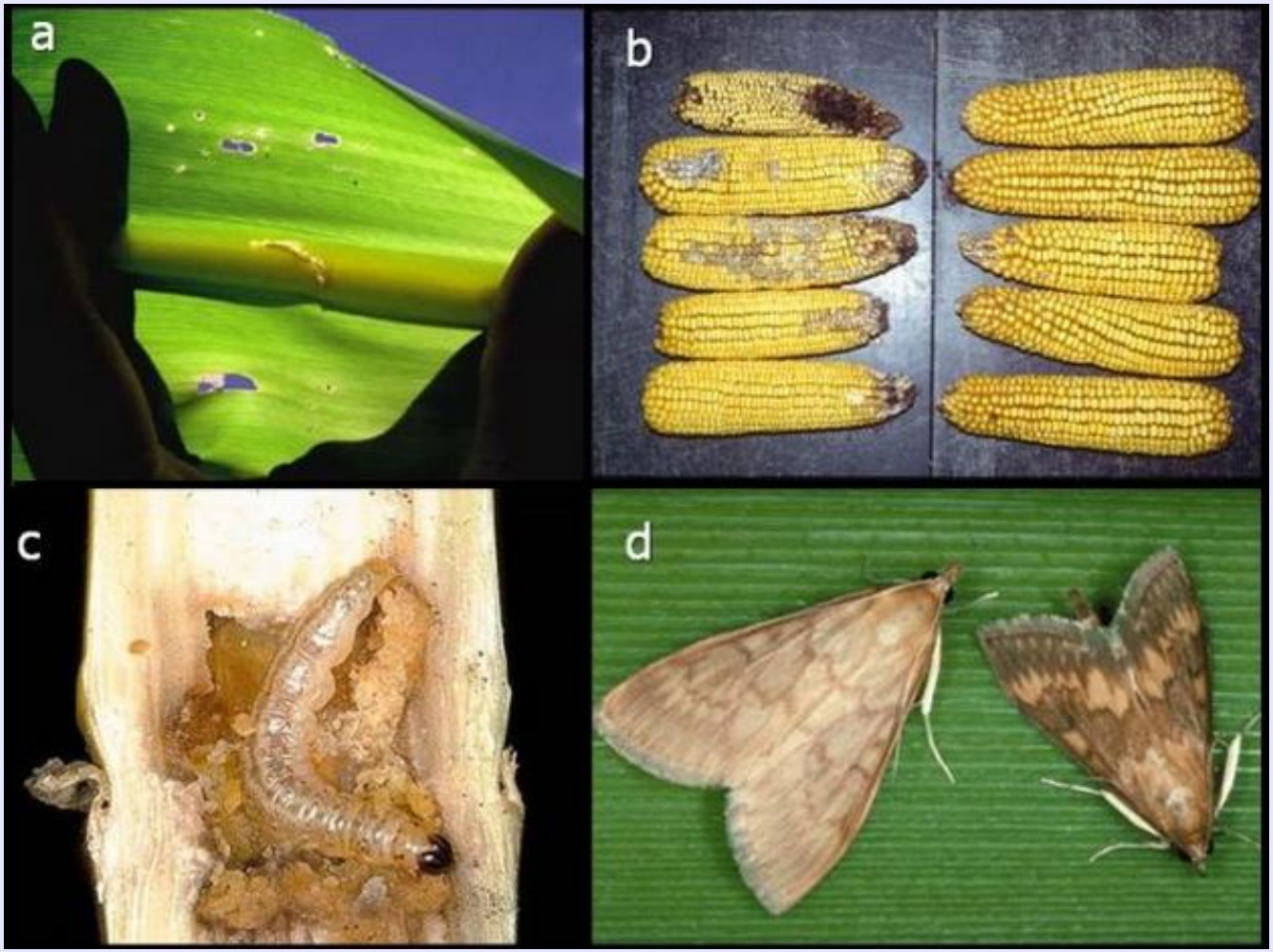


Image: Hellmich & Hellmich (2012) Nature Education Knowledge 3(10):4

`http://www.nature.com/scitable/content/ne0000/ne0000/ne0000/ne0000/46977030/1_2.jpg`

It does not take a village to publish a genome!

- In the past:
  - Human: International Human Genome Sequencing Consortium (2001), Nature 409(6822), 860 **248 authors**
  - Mosquito: Nene et. al (2007) **95 authors**

It does not take a village to publish a genome!

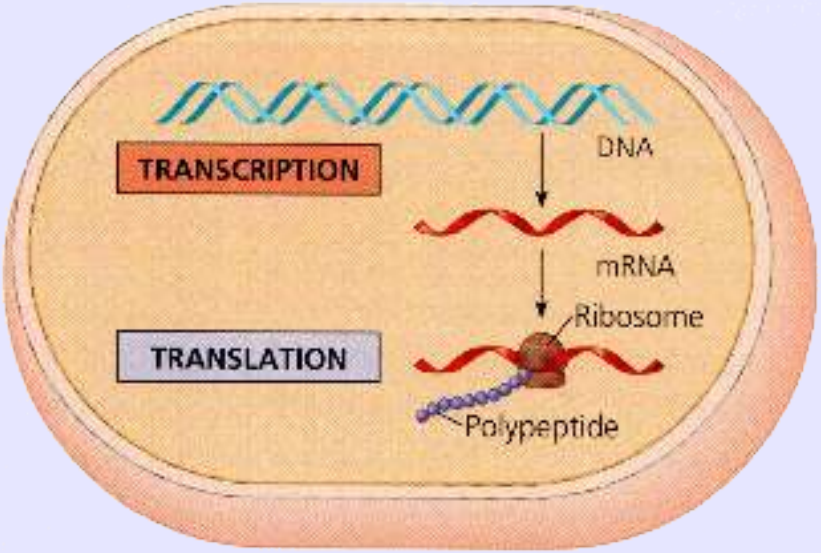
- In the past:
  - Human: International Human Genome Sequencing Consortium (2001), Nature 409(6822), 860 **248 authors**
  - Mosquito: Nene et. al (2007) **95 authors**
- More recently:
  - 4 *Botrytis cinerea*: Adhikari et al. (2025), **5 authors**
  - European harvest mouse: O'Brien & Colom (2024), **2 authors**
  - Great wood-rush: Goodwin et al. (2024), **4 authors**

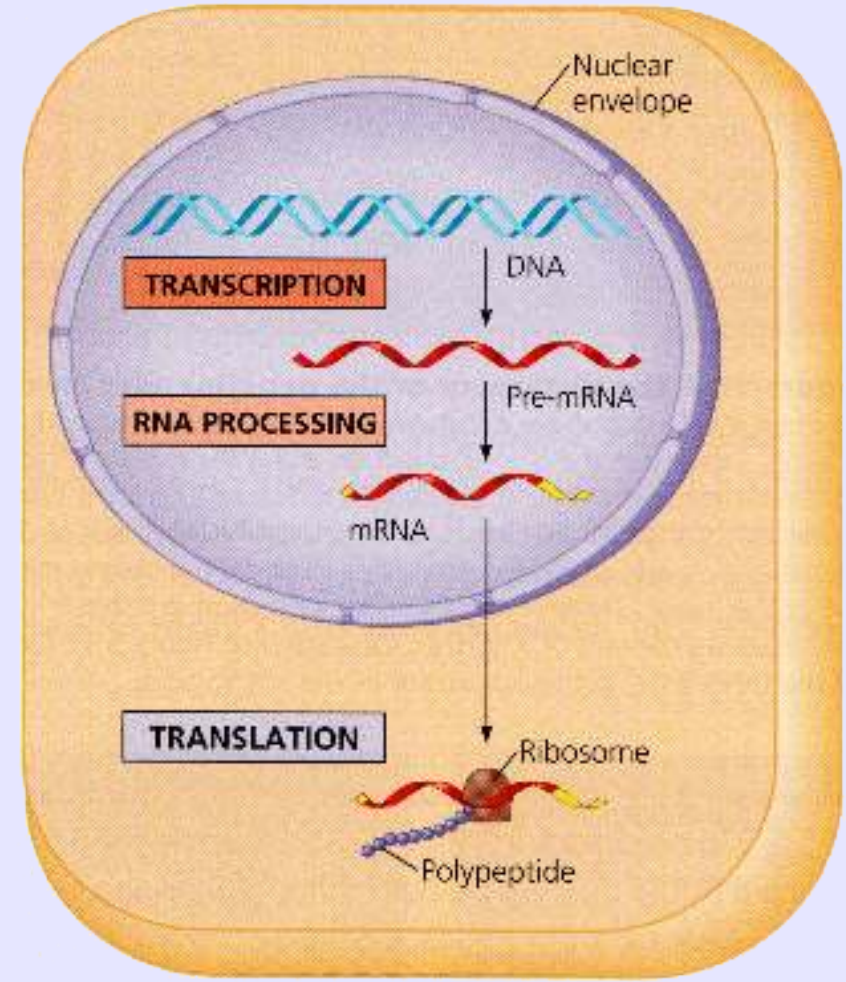
It does not take a village to publish a genome!

- In the past:
  - Human: International Human Genome Sequencing Consortium (2001), Nature 409(6822), 860 **248 authors**
  - Mosquito: Nene et. al (2007) **95 authors**
- More recently:
  - 4 *Botrytis cinerea*: Adhikari et al. (2025), **5 authors**
  - European harvest mouse: O'Brien & Colom (2024), **2 authors**
  - Great wood-rush: Goodwin et al. (2024), **4 authors**
- **You can do it!**

How does a cell recognize protein-coding genes?

Transcription & Translation





Images: Campbell et al., Biology, San Francisco, 2008, p. 329, Fig. 17.3

How does a cell recognize protein-coding genes?

Prokaryotes & Eukaryotes*

How does a cell recognize protein-coding genes?

Prokaryotes & Eukaryotes*

*) only some of the genes in eukaryotes

How does a cell recognize protein-coding genes?

Prokaryotes & Eukaryotes*

- every protein coding gene has an ORF
- not every ORF is a protein coding gene

How does a cell recognize protein-coding genes?

Eukaryotes: Splicing of introns

The Genome Annotation Problem

Genomic Sequence: chicken

cctcacctctgagaaaacctctttgccaccaataccatgaagctctgcgtgactgtcctgtctctcctc
gtgctagtagctgccttctgctctctagcactctcagcaccaagtaagtctacttttgcagctgctatt
tcgagtcaaggtgtaggcagagtccttttttctagtcatggctggcaaacagtgggatctggggatggg
acaaaaggcagctaggaagattgccatgtagtctgctgctaaatgtagagtctagtagatattcagtaa
cattcaagttcctattttcttaagaattagcaaccagcagaggaaaacgatgggctggaagtcagactg
ttgaattggctctgcctttaattatttgttcaagcaagcccctgtccctctctgtgccttggtttcccc
atctgtcatatgaagggagtgcgatgtgttctgagactgaatccagttccaatcttctagatttctttc
tcgttcttctctgaagatccactattcagaataagactcctgctcatgttaggtgggaatggatacaag
ggaccatatttggggttctggtagctccacagggatgctcaatgaagatgcaaaattagaagtcaaaat
aaacagctcccatgggcagtgttgatctcaccctggcctttcctttcagtgggctcagaccctcccacc
gcctgctgcttttcttacaccgcgaggaagcttcctcgcaactttgtggtagattactatgagaccagc
agcctctgctcccagccagctgtggtgtgagtatcaacccctggctgccctgggaggcaagggtgaggg
ctggatttttaaaggggcctgttttggggaggggtgatgagcgctggggaggcagctctcagggctg
aagccttccctgacagcagtgaggtcacaggtcatgaactcacttttcaagtgctgaaggcggctgagt
ggcagccgagacagaaggggttcctggggaggaagttattcagaggacagggaagcaggggaaggcag
acaggtcccatgagatatggaccaattccttaaaccatgctagaaaaacatgtggaaaagtcactacca
ggctggcagggaatggggcaatctattcatactgattgcaatgcccactggttcctaatctgggcaacc
cctggggcccacagctaaatccagtgagtggaagttacagggagtctgcttccagtgctgctcgaggaa
ggatcccatccaccagagctgccccacatggaccatggtcaggcagaggaagatgcctaccacaggcaa
gggataaagccagatgacctcaaaggtcccatgggattctaatctgtctgctccttgttctacagattc
caaaccaaaagaggcaagcaagtctgcgctgaccccagtgagtcctgggtccaggagtacgtgtatgac
ctggaactgaactgagctgctcagagacaggaagtcttc

The Genome Annotation Problem

Genomic sequence: chicken (1 gene: macrophage inflammatory protein-1 b)

cctcacctctgagaaaacctctttgccaccaataccatgaagctctgcgtgactgtcctgtctctcctc
gtgctagtagctgccttctgctctctagcactctcagcaccaagtaagtctacttttgcagctgctatt
tcgagtcaaggtgtaggcagagtccttttttctagtcatggctggcaaacagtgggatctggggatggg
acaaaaggcagctaggaagattgccatgtagtctgctgctaaatgtagagtctagtagatattcagtaa
cattcaagttcctattttcttaagaattagcaaccagcagaggaaaacgatgggctggaagtcagactg
ttgaattggctctgcctttaattatttgttcaagcaagcccctgtccctctctgtgccttggtttcccc
atctgtcatatgaagggagtgcgatgtgttctgagactgaatccagttccaatcttctagatttctttc
tcgttcttctctgaagatccactattcagaataagactcctgctcatgttaggtgggaatggatacaag
ggaccatatttggggttctggtagctccacagggatgctcaatgaagatgcaaaattagaagtcaaaat
aaacagctcccatgggcagtgttgatctcaccctggcctttcctttcagtgggctcagaccctcccacc
gcctgctgcttttcttacaccgcgaggaagcttcctcgcaactttgtggtagattactatgagaccagc
agcctctgctcccagccagctgtggtgtgagtatcaaccctggctgccctgggaggcaagggtgaggg
ctggatttttaaaggggcctgttttggggaggggtgatgagcgctggggaggcagctctcagggctg
aagccttccctgacagcagtgaggtcacaggtcatgaactcacttttcaagtgctgaaggcggctgagt
ggcagccgagacagaaggggttcctggggaggaagttattcagaggacagggaagcaggggaaggcag
acaggtcccatgagatatggaccaattccttaaaccatgctagaaaaacatgtggaaaagtcactacca
ggctggcagggaatggggcaatctattcatactgattgcaatgcccactggttcctaatctgggcaacc
cctggggcccacagctaaatccagtgagtggaagttacagggagtctgcttccagtgctgctcgaggaa
ggatcccatccaccagagctgccccacatggaccatggtcaggcagaggaagatgcctaccacaggcaa
gggataaagccagatgacctcaaaggtcccatgggattctaatctgtctgctccttgttctacagattc
caaaccaaaagaggcaagcaagtctgcgctgaccccagtgagtcctgggtccaggagtacgtgtatgac
ctggaactgaactgagctgctcagagacaggaagtcttc

What aids in the identification of genes in genomes?

Evidence data from transcription

What aids in the identification of genes in genomes?

Evidence data from translation

What aids in the identification of genes in genomes?
Mathematical models

What aids in the identification of genes in genomes?
Mathematical models

Simplification

- There are only 2 nucleotides: A, B
- There are only 2 sequence states: intergenic (I), coding sequence (K)

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Markov property

1. The current value of the hidden state depends exclusively on the state of its predecessor.

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Model assumptions

1. The current value of the hidden state depends exclusively on the state of its predecessor.
2. The current value of the visible observation depends exclusively on the value of the current, hidden state.

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Basis of highly accurate gene prediction tools

Hidden Markov Model

Find the most probable hidden state sequence for a given sequence

Idea:

1. Generate all possible state sequences
2. Calculate the probability for each state sequence
3. Choose the state sequence with the highest probability

$\Rightarrow$ too expensive!

Basis of highly accurate gene prediction tools

Hidden Markov Model

Hidden Markov Model for gene identification in practice

EBI: Ensembl annotation system

Ensembl annotation pipelines

Ensembl annotation pipeline for non-vertebrates

Pipelines

- Ensembl vertebrate pipeline: `https://rapid.ensembl.org/info/genome/genebuild/full_genebuild.html`
- Ensembl non-vertebrate pipeline: `https://rapid.ensembl.org/info/genome/genebuild/anno.html`
- BRAKER2 in Ensembl: `https://rapid.ensembl.org/info/genome/genebuild/braker.html`

Where to find annotations

- ENSEMBL: `https://beta.ensembl.org/`

Notes by Katharina

- Can (probably) only be installed and executed by EBI
- Not publicly benchmarked against other pipelines

Genome Annotation at the NCBI

Different Annotation Scenarios

- Internal: The NCBI Eukaryotic Genome Annotation Pipeline (EGAP)
- (Internal: RefSeq curation)
- You can run it: **EGAPx**

Annotation with EGAPx (NCBI)

Annotation with EGAPx (NCBI)

- Containerized with Docker/Singularity
- Documentation: `https://github.com/ncbi/egapx`
- Currently supported clades (protein sets):
  - Chordata
  - Insecta
  - Arthropoda
  - Monocots
  - Eudicots
- Easy to use
- Benchmarking possible: good accuracy!

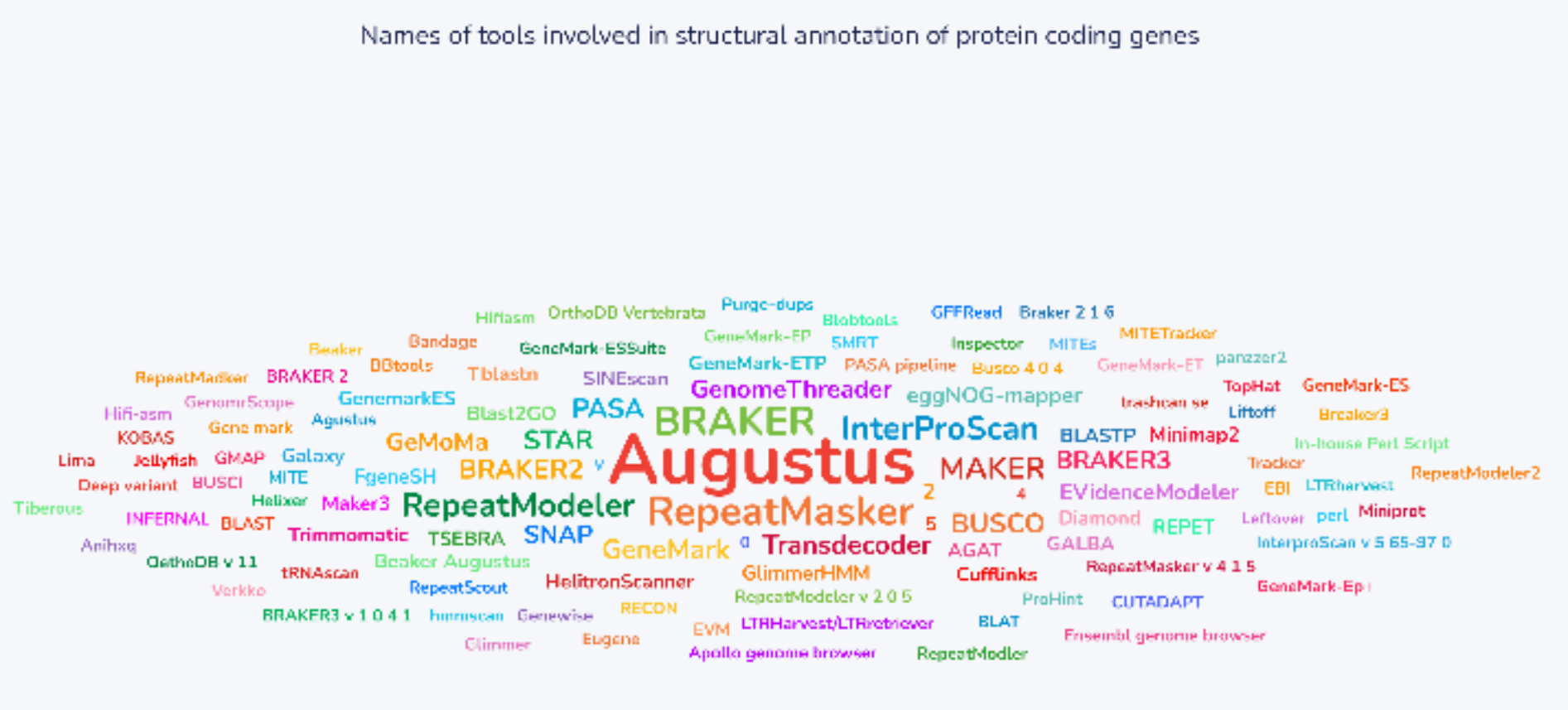
Read your methods snippet

Focus on structural annotation of protein coding genes only!

1. We move to Wooclap
2. Enter the names of tools involved in structural annotation of protein coding genes

Read your methods snippet

Focus on structural annotation of protein coding genes



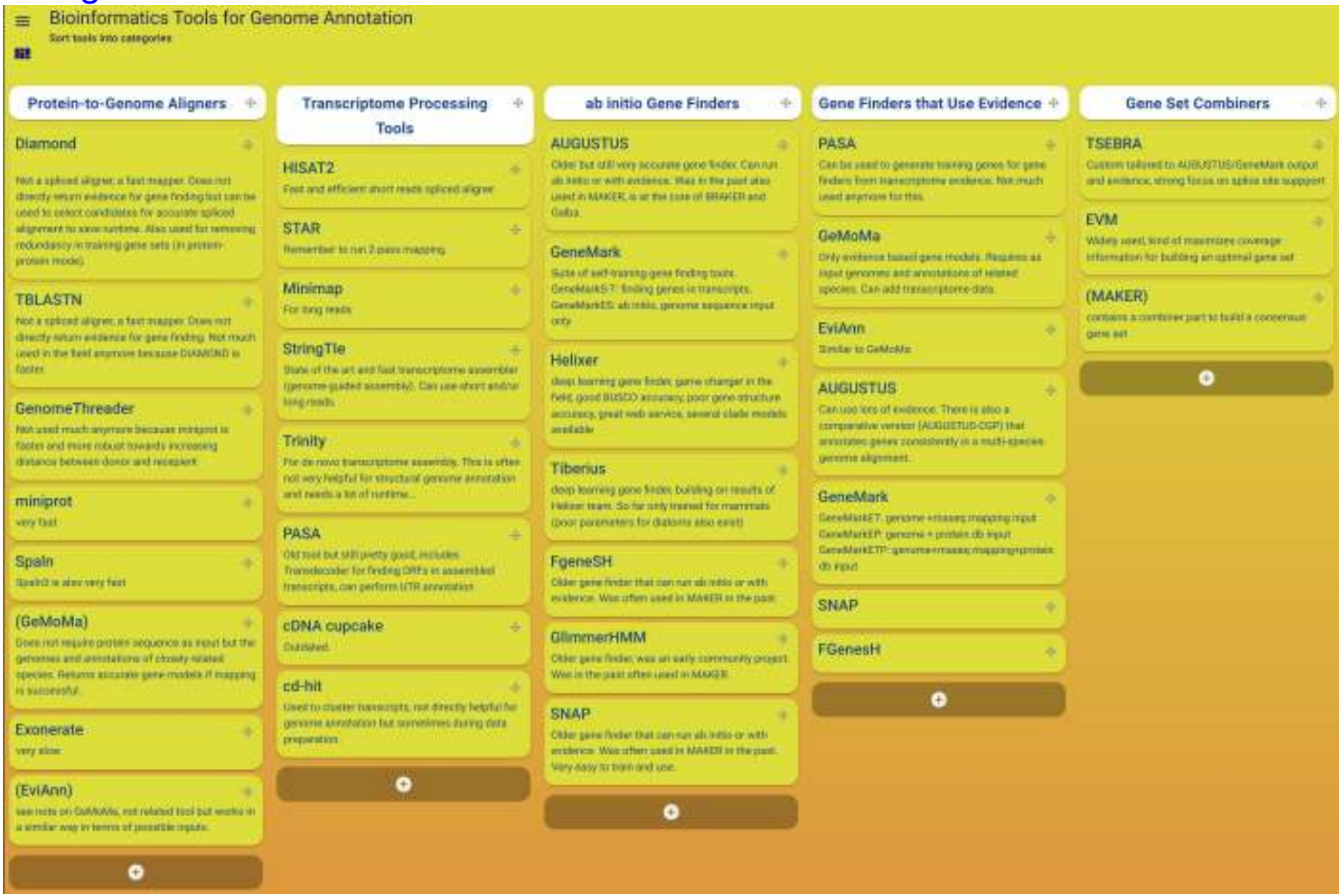
Categorize tool names

Go to

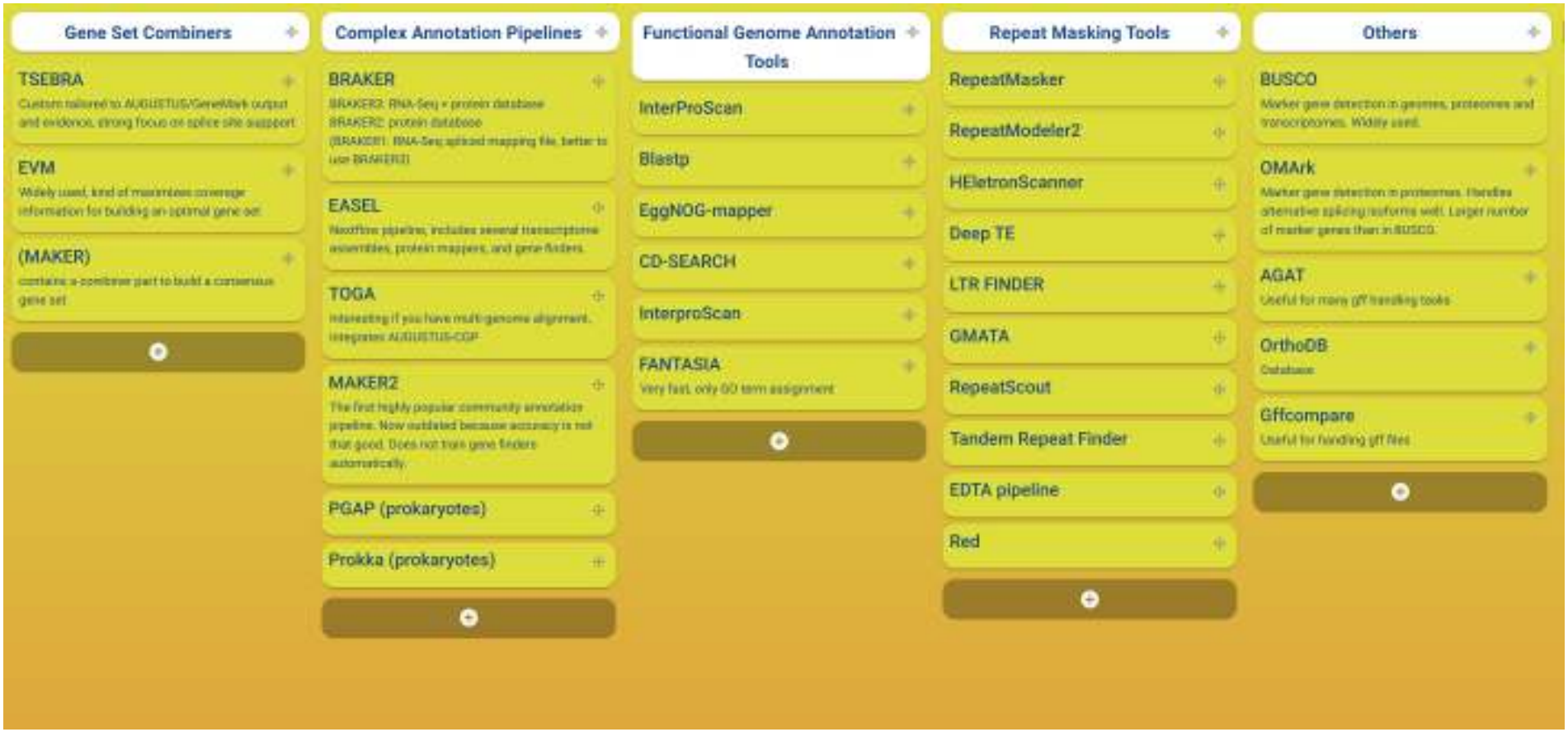
`https://shorturl.at/uA0Tg`

and sort the tools names from your methods snippet into categories

Categorize tool names



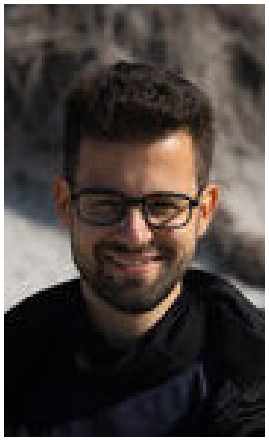
Categorize tool names



The aera of genome annotation super heroes

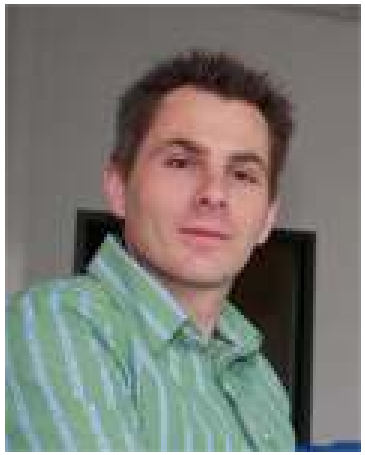
The BRAKER Team

University of Greifswald & Georgia Tech University

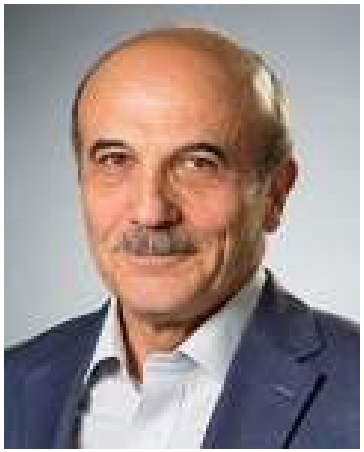


Lars Gabriel

Alexandre Lomsadze, Katharina Hoff, Tomáš Brůna



Mario Stanke



Mark Borodovsky

Also: Simone Lange, Matthis Ebel, Hannah Thierfeldt, Anica Hoppe, Neng Huang

BRAKER1: Unsupervised RNA-Seq-Based Genome Annotation with GeneMark-ET and AUGUSTUS

Katharina J. Hoff ✉, Simone Lange, Alexandre Lomsadze, Mark Borodovsky ✉, Mario Stanke

Bioinformatics, Volume 32, Issue 5, 1 March 2016, Pages 767–769,
https://doi.org/10.1093/bioinformatics/btv661

- spliced alignments of RNA-Seq are used by GeneMark-ET and AUGUSTUS
- 1,677 citations (Google Scholar)

Whole-Genome Annotation with BRAKER

Katharina J. Hoff, Alexandre Lomsadze, Mark Borodovsky, and Mario Stanke

in Kollmar M. (eds) Gene Prediction. Methods in Molecular Biology, vol 1962. Humana, New York, NY, 2019

GeneMark-ET uses RNA-Seq for Training

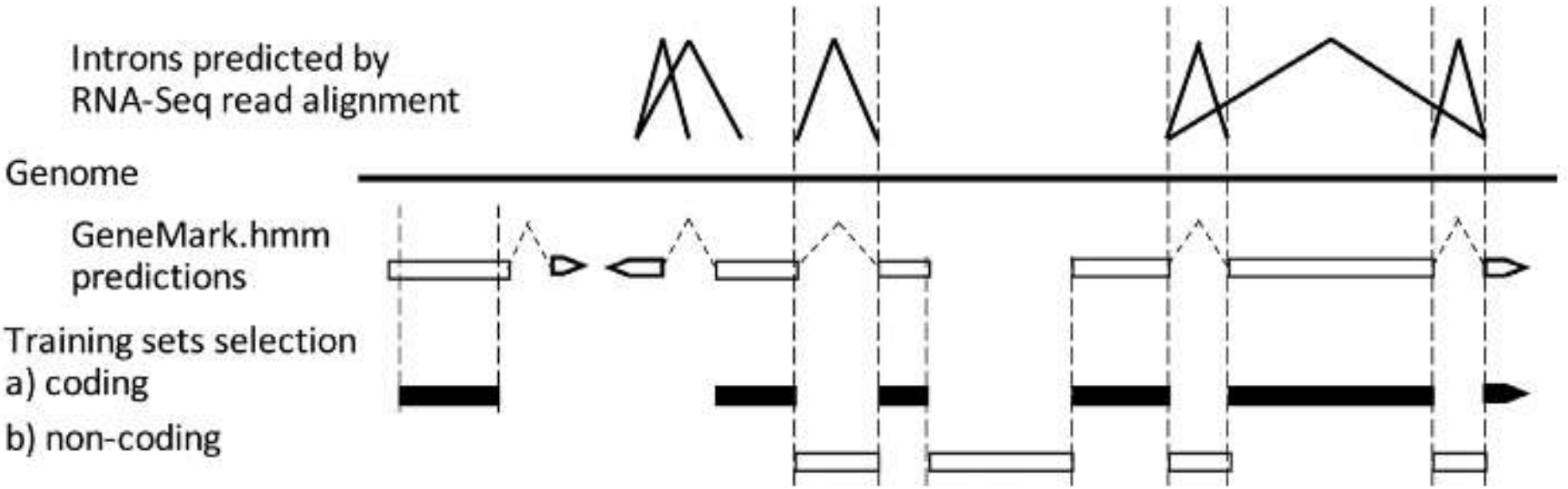


Figure 3. Selection of elements of training set in GeneMark-ET for the next iteration. The new training set of protein-coding regions is comprised from exons with at least one 'anchored splice site' as well as long exons predicted *ab initio* (>800 nt).

- employs unsupervised training
- training includes introns and exons anchored by mapped RNA-Seq reads
- does not require RNA-Seq reads assembly
- does not use RNA-Seq information in the *prediction* step

AUGUSTUS – RNA-Seq for Prediction

- requires “prior data” for training
- uses intron information from RNA-seq for *prediction*
- no RNA-Seq assembly required
- optional input: BUSCO lineage (compleasm)

Measuring accuracy of genome annotation

Experiment

Accuracy assessment after applying tool to genome with reference annotation:

Species	Genome Size (Mb)	# Genes in Annotation
Arabidopsis thaliana (thale cress)	119	27,444
Bombus terrestris (bumble bee)	249	10,581
Caenorhabditis elegans (nematode)	100	20,172
Danio rerio (zebrafish)	1,345	25,611
Drosophila melanogaster (fruit fly)	137	13,928
Gallus gallus (chicken)	1,040	17,279
Medicago truncatula (barrelclover)	420	44,464
Mus musculus (mouse)	2,650	22,378
Parasteatoda tepidariorium (house spider)	1,445	18,602
Populus trichocarpa (poppy)	389	34,488
Solanum lycopersicum (tomato)	772	33,562

Accuracy metrics

Precision = Specificity: Percentage of correctly found genes/transcripts/exons in the **predicted gene set**.

Recall = Sensitivity: Percentage of correctly found genes/transcripts/exons in the **reference annotation**.

F1-Score: $$\frac{2 \cdot \text{Recall} \cdot \text{Precision}}{\text{Recall} + \text{Precision}}$$

BRAKER1 gene F1 accuracy

Use only if not enough RNA-Seq for BRAKER3!

Published online 6 January 2021 *NAR Genomics and Bioinformatics, 2021, Vol. 3, No. 1 1*
doi: 10.1093/nargab/lqaa108

BRAKER2: automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database

Tomáš Brůna[1,†], Katharina J. Hoff[2,3,†], Alexandre Lomsadze[4], Mario Stanke[2,3,‡] and Mark Borodovsky[4,5,*,†]

- spliced alignments of a large number of proteins (e.g. OrthoDB partition)
- optional input: BUSCO lineage (compleasm)
- 1,269 citations (Google Scholar)

BRAKER2 gene F1 accuracy

Use only if you have no RNA-Seq data on genomes <1 Gbp

BRAKER3 gene F1 accuracy - climbing the top

BRAKER3: using RNA-Seq and protein evidence with GeneMark-ETP, AUGUSTUS and TSEBRA

- Gabriel *et al.* (2024)
- 137 citations (Google Scholar)
- spliced aligned and **assembled** RNA-Seq
- large protein database
- optional input: BUSCO lineage (compleasm)
- combines GeneMark-ETP and AUGUSTUS gene sets with TSEBRA

BRAKER3: using RNA-Seq and protein evidence with GeneMark-ETP, AUGUSTUS and TSEBRA

Gabriel et al. BMC Bioinformatics (2021) 22:566
https://doi.org/10.1186/s12859-021-04482-0

BMC Bioinformatics

SOFTWARE Open Access

TSEBRA: transcript selector for BRAKER

Lars Gabriel[1,2], Katharina J. Hoff[1,2], Tomáš Brůna[3], Mark Borodovsky[4,5] and Mario Stanke[1,2*]

- **combines** several gene sets according to evidence
- 154 citations (Google Scholar)

Can be used to combine BRAKER1 and BRAKER2 output if BRAKER3 fails.

Accuracy of genome annotation approaches by BRAKER team

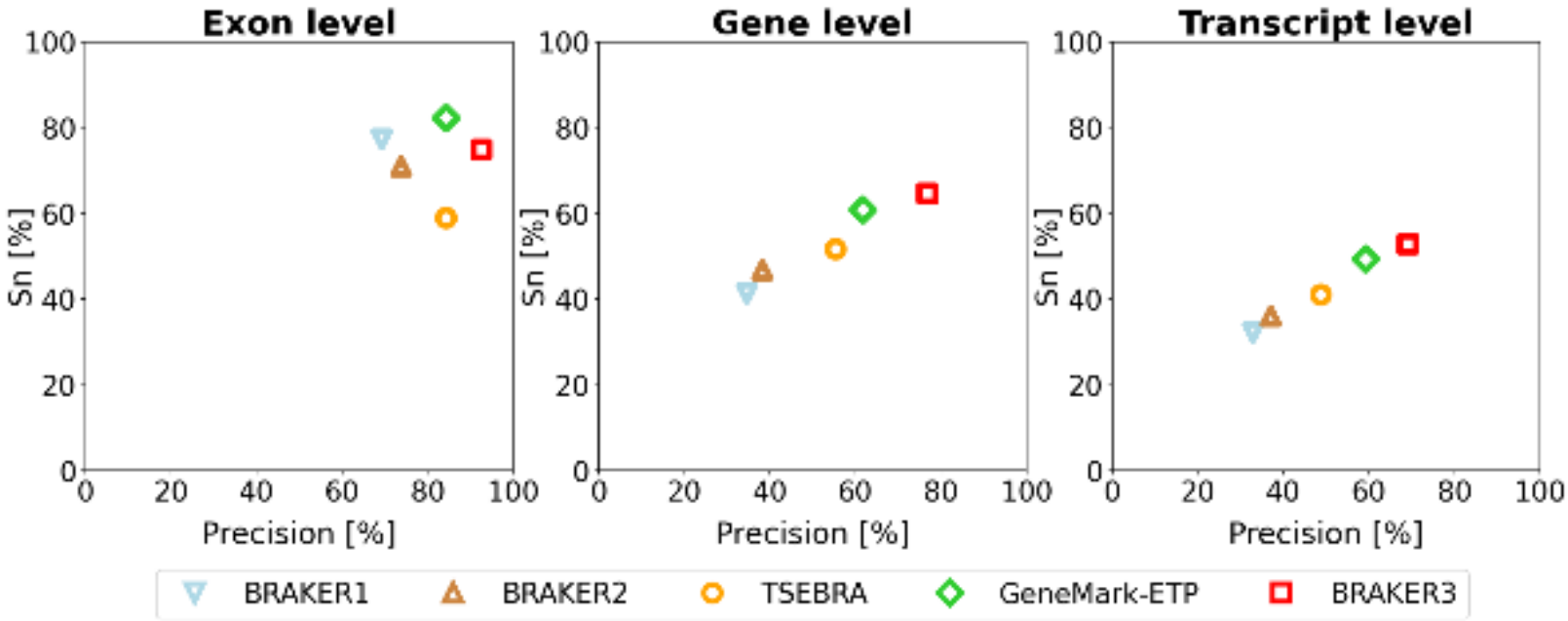


Figure 2. Average precision and sensitivity of gene predictions made by BRAKER1, BRAKER2, TSEBRA, GeneMark-ETP, and BRAKER3 for the genomes of 11 different species (listed in Supplemental Table S1). Inputs were the genomic sequences, short-read RNA-seq libraries, and protein databases (*order excluded*).

Image: Gabriel *et al.* (2024), Genome Research

Availability

GitHub

```
https://github.com/Gaius-Augustus/BRAKER
```

Docker/Singularity

```
singularity build braker.sif \
        docker://teambraker/braker:latest

singularity exec braker.sif braker.pl [OPTIONS]
```

License

- BRAKER: Artistic License
- most components unter open source software licenses
- GeneMark-ETP: CC BY-NC

GALBA Contributors

Tomáš Brůna

Heng Li

Joseph Guhlin

Lars Gabriel

Natalia Nenasheva

Ethan Tolman

Paul Frandsen

Matthis Ebel

Mario Stanke

Katharina Hoff

Brunet et al. BMC Bioinformatics (2025) 26:227
https://doi.org/10.1186/s12859-025-06179-z

BMC Bioinformatics

RESEARCH Open Access

Galba: genome annotation with miniprot and AUGUSTUS

- 28 citations (Google Scholar)
- for genomes >1Gbp
- proteins of close relatives

Proteins Only (GALBA, BRAKER2, FunAnnotate) vs. BRAKER3 with RNA-Seq & Proteins

GALBA: Gene F1 Accuracy

If you have RNA-Seq, use BRAKER3!

Availability

GitHub

```
https://github.com/Gaius-Augustus/GALBA
```

Docker/Singularity

```
singularity build galba.sif \
        docker://katharinahoff/galba:latest

singularity exec galba.sif galba.pl [OPTIONS]
```

License

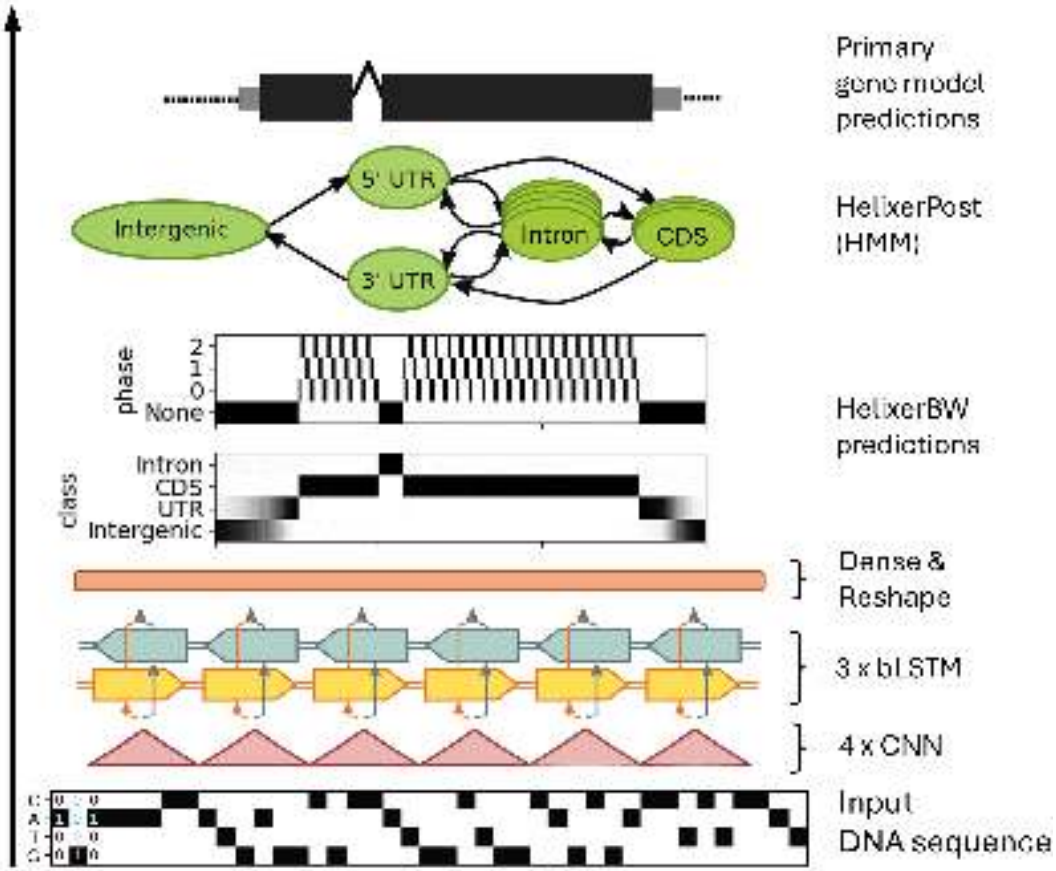
- GALBA: Artistic License
- all dependencies have Open Source Licenses

Helixer: bringing deep learning into genome annotation

Image: ChatGPT by OpenAI, manual editing

HELIXER–*de novo* PREDICTION OF PRIMARY EUKARYOTIC GENE MODELS COMBINING DEEP LEARNING AND A HIDDEN MARKOV MODEL

Felix Holst[1†], Anthony Bolger[2†], Christopher Günther[1], Janina Maß[3], Sebastian Triesch[1,4], Felicitas Kindel[1], Niklas Kiel[1,4], Nima Saadat[3,4], Oliver Ebenhöh[3,4], Björn Usadel[2,4,5], Rainer Schwacke[2], Marie Bolger[2], Andreas P.M. Weber[1,4], Alisandra K. Denton[1,4]



- 27 citations (Google Scholar)
- cross-species gene finder
- *ab initio* prediction
- Pre-trained models for:
  - fungi
  - land plant
  - vertebrate
  - invertebrate
- accuracy (BUSCO): good
- web service

Availability: `https://github.com/weberlab-hhu/Helixer`

Image of Helixer: `https://github.com/weberlab-hhu/Helixer/blob/main/img/network.png`

Tiberius: improved genome annotation with deep learning

Image: ChatGPT by OpenAI, manual editing

The Tiberius Team

University of Greifswald

Lars Gabriel, Felix Becker, Katharina Hoff, Mario Stanke

Tiberius: end-to-end deep learning with an HMM for gene prediction

Lars Gabriel [1,2,*], Felix Becker [1,2], Katharina J. Hoff[1,2], Mario Stanke [1,2,*]

- builds on findings by Helixer team
- cross-species gene finder
- faster
- higher accuracy
- *ab initio* prediction
- Pre-trained model(s) for:
  - mammals
  - (diatoms)
- container for A100 GPU

Availability: `https://github.com/Gaius-Augustus/Tiberius`

Accuracy of state of the art gene finders

No alternative splicing isoforms

Image: Lars Gabriel, PAG presentation 2025

Current shortcomings of deep learning gene finders

- no evidence integration
- no alternative splicing isoform prediction
- require expensive GPU for feasible runtime
- limited to specific clades

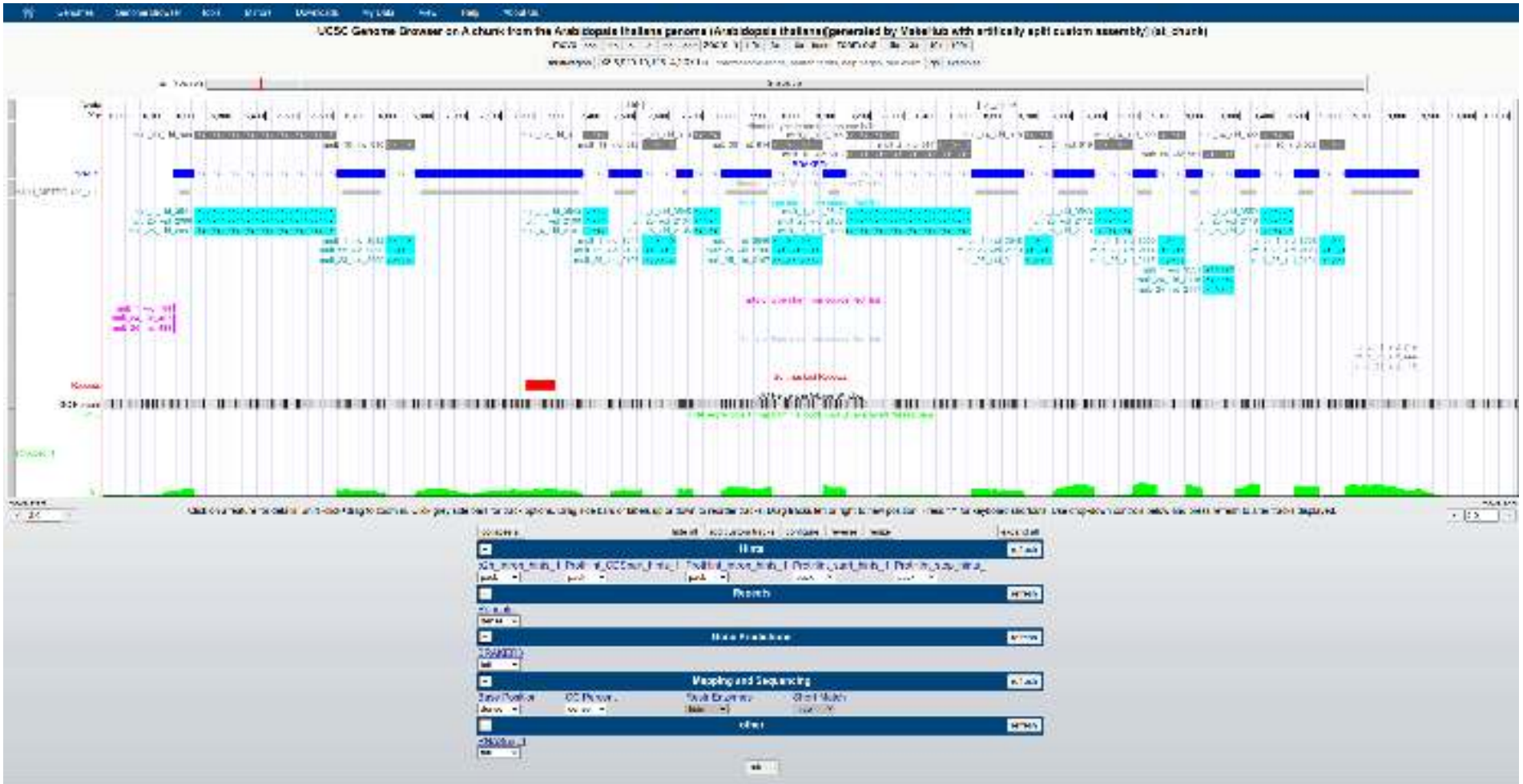
$\rightarrow$ BRAKER3, Galba & EGAPx currently remain important

Did We Do a Good Job?

Genome Browsers

Visualize your Annotation in Context with Evidence

- UCSC Genome Browser, MakeHub
- JBrowse
- ...

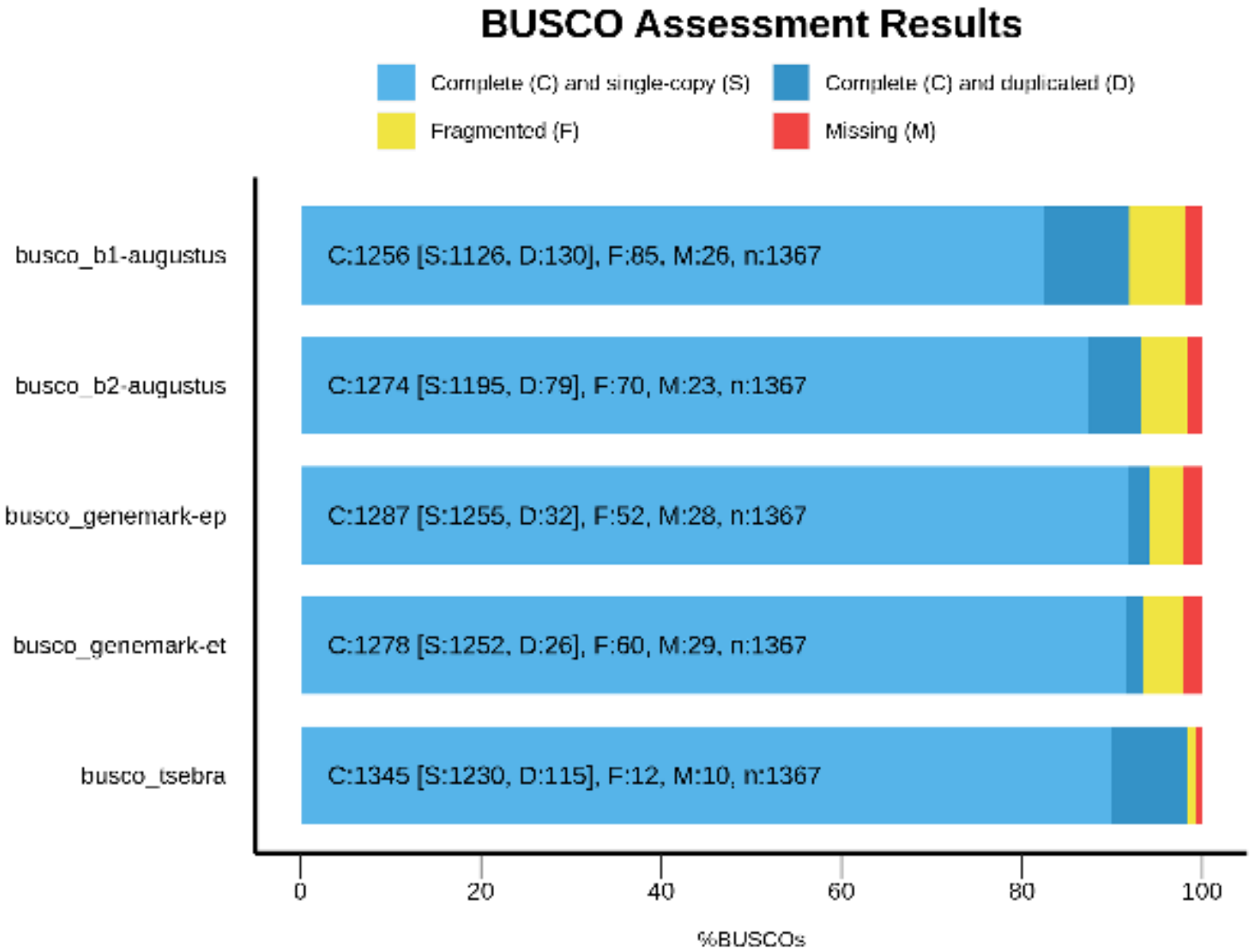


Describe Your Annotation

- number of genes
- number of transcripts
- ratio of mono-exonic to multi-exonic genes
- median number of exons per transcript
- maximal number of exons per transcript
- median transcript length
- ...

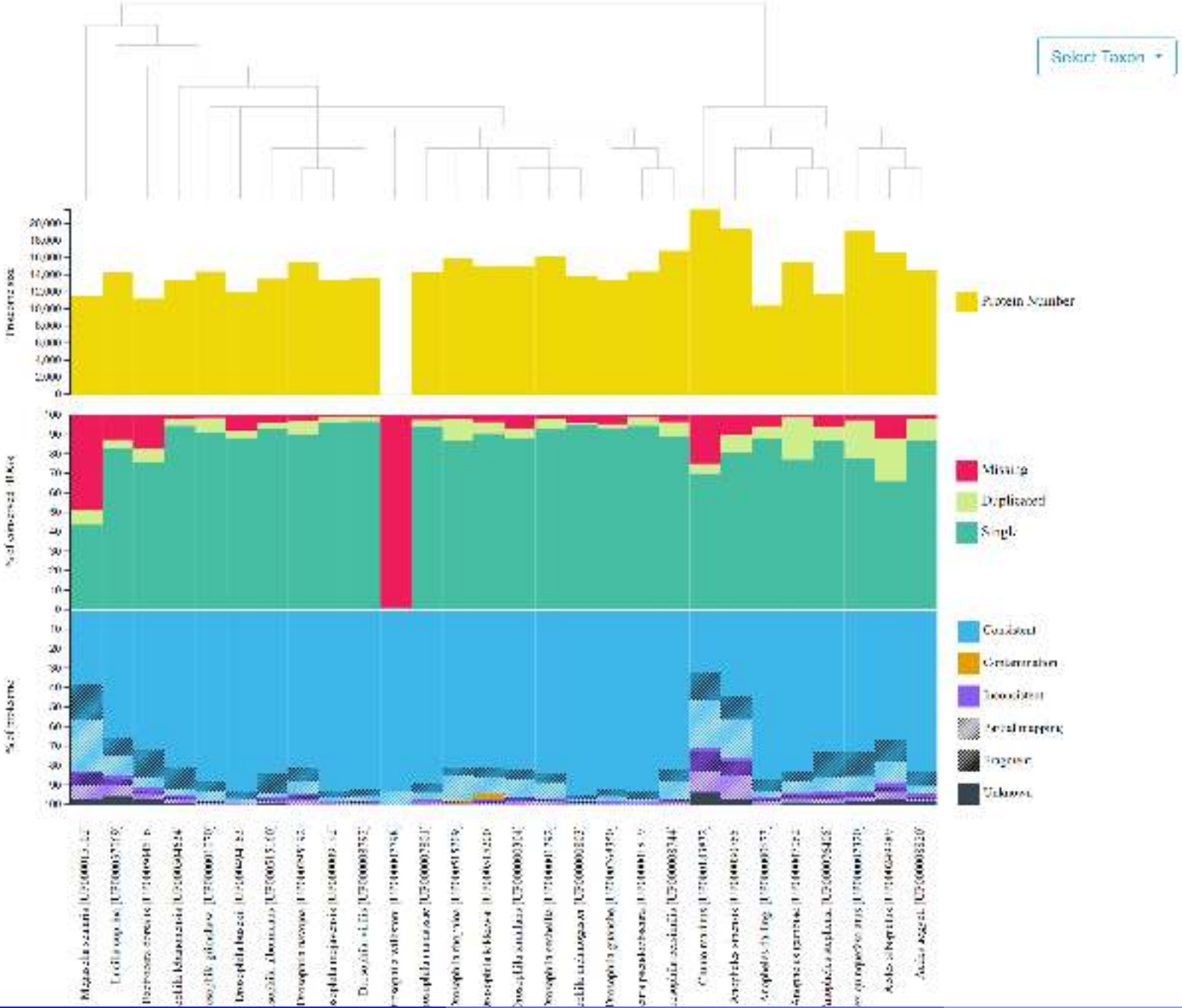
If possible, compare to annotated close relatives.
Consider effect of individual annotation pipelines.

BUSCO: Sensitivity in Clade-Specific Conserved Genes



Beware! BUSCO completeness does not warrant correct gene structures!

OMArk: Sensitivity, Contaminations, & More



Revisiting annotation methods sections

1. Read your methods snippet, again
2. Use our categorized tool name board at `https://shorturl.at/uA0Tg` if you are still unsure what a tool does
3. Ask if you remain unsure what a method is good for
4. Fill the poll on Wooclap

Revisiting annotation methods sections

Most important stuff on genome annotation

- structural genome annotation in eukaryotes is hard
- Hidden Markov Models are essential
- evidence helps a lot
- majority of genomes is annotated by large centers
- popular community annotation pipelines:
  1. BRAKER
  2. GALBA
  3. (EGAPx may become popular)
- deep learning is changing the field
  1. Helixer (careful with accuracy)
  2. Tiberius (only for two clades)
- "looking nice" is not always "correct"
- BUSCO completeness is widely used
- OMArk might be more appropriate
- high marker gene detection rate $\neq$ high accuracy