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BRAKER2: Proteins

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Structural Genome Annotation with BRAKER3

BGA23 Workshop

Katharina J. Hoff

Contact: katharina.hoff@uni-greifswald.de

Eukaryotic Gene

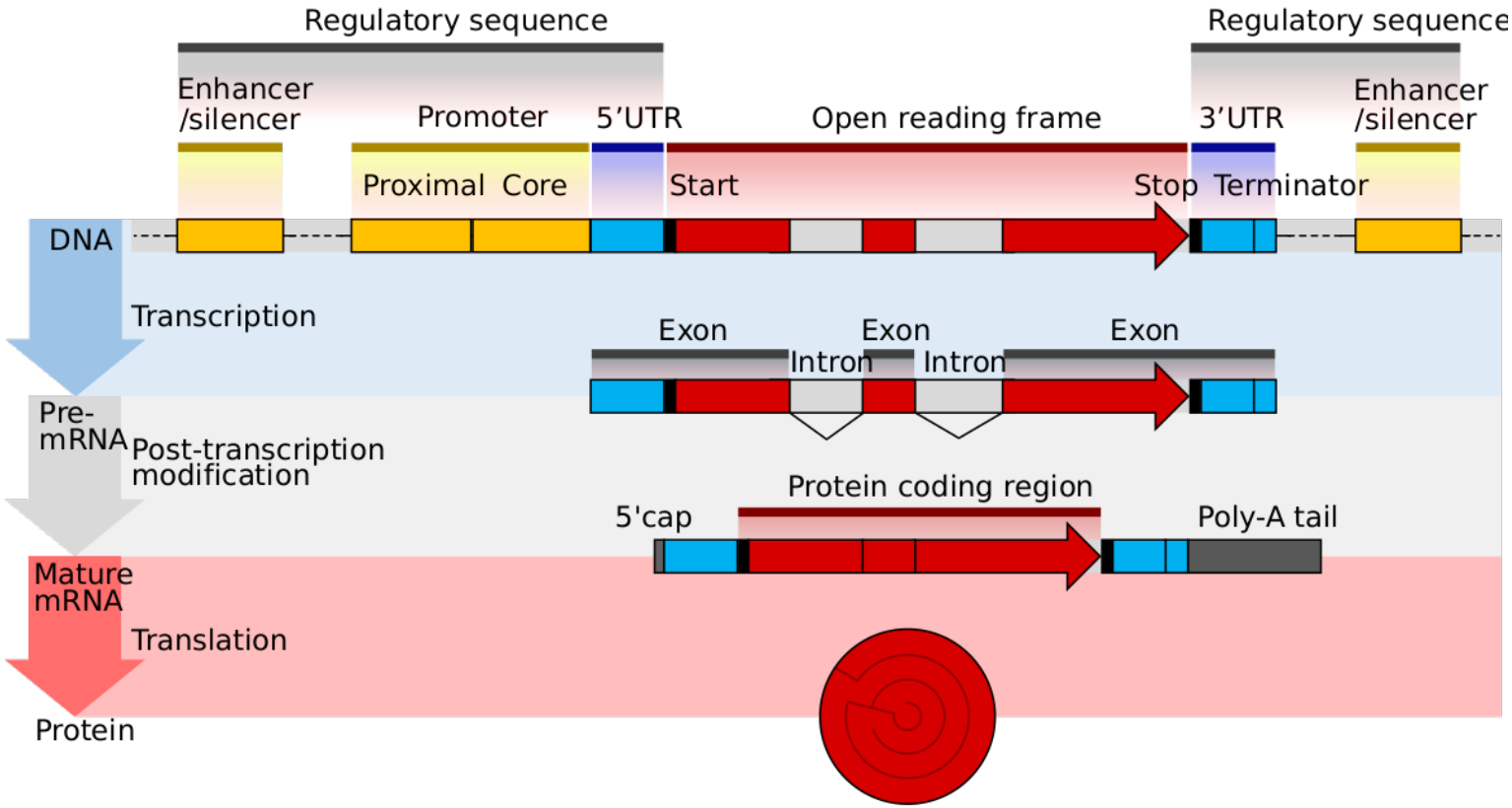
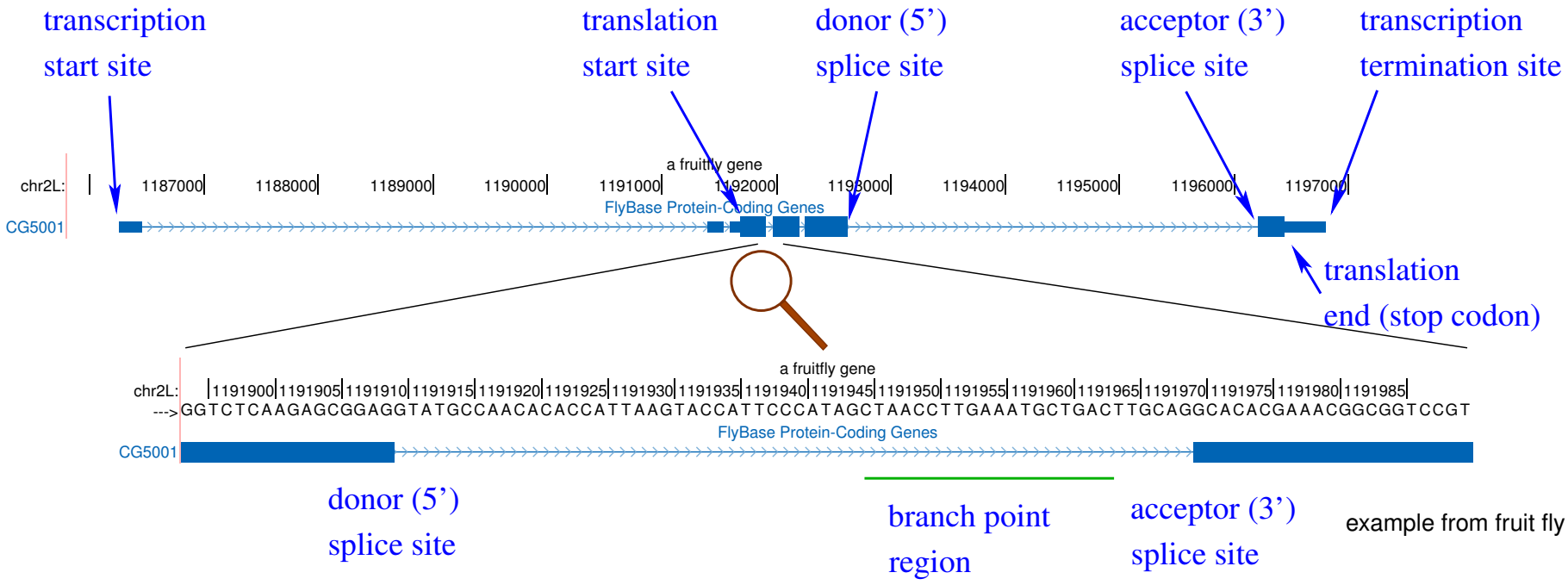


Image: Wikipedia, CC BY 4.0

Information for Genome Annotation

- genome sequence: **mathematical model**
- observed mRNA expression (parts)
- known proteins of relatives

Signals



Sequence Content

Besides the signals, position-unspecific frequencies of nucleotide patterns (e.g. k-mer frequencies) can be used to guess biological classification of longer sequence intervals.

$\Rightarrow$ species specific parameters $\Rightarrow$ need training $\Rightarrow$ need training examples

Structural Genome Annotation Problem

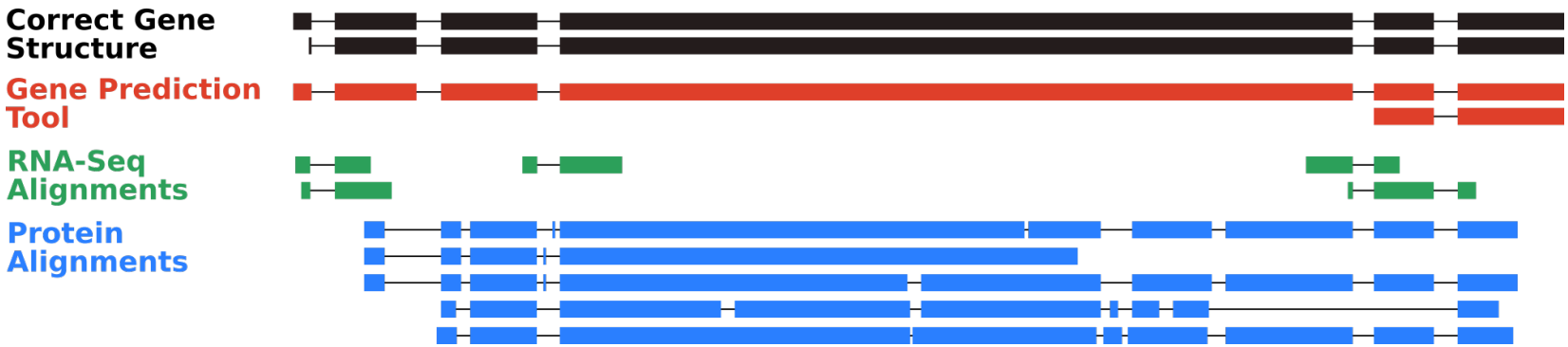
Input

- genome assembly
- extrinsic evidence, e.g. from RNA-Seq, protein sequences

Output

- protein-coding genes: CDSexon-intron structures (`.gff`)

Task: find and predict gene structures of protein-coding genes



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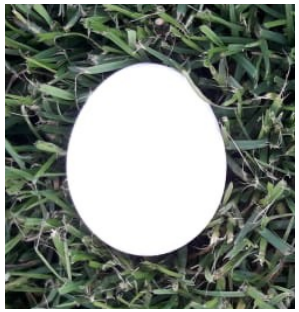
Gene Finders Need Training Examples



Genes
(Parameter training)

Genes
(Prediction)

Katharina J. Hoff

UNIVERSITÄT GREIFSWALD
Wissen lockt. Seit 1456

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Gene Finders Need Training Examples

Image: credits to DALL-E2, modified by human

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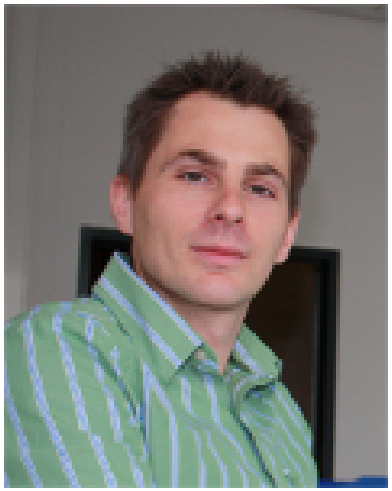
The BRAKER Team

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

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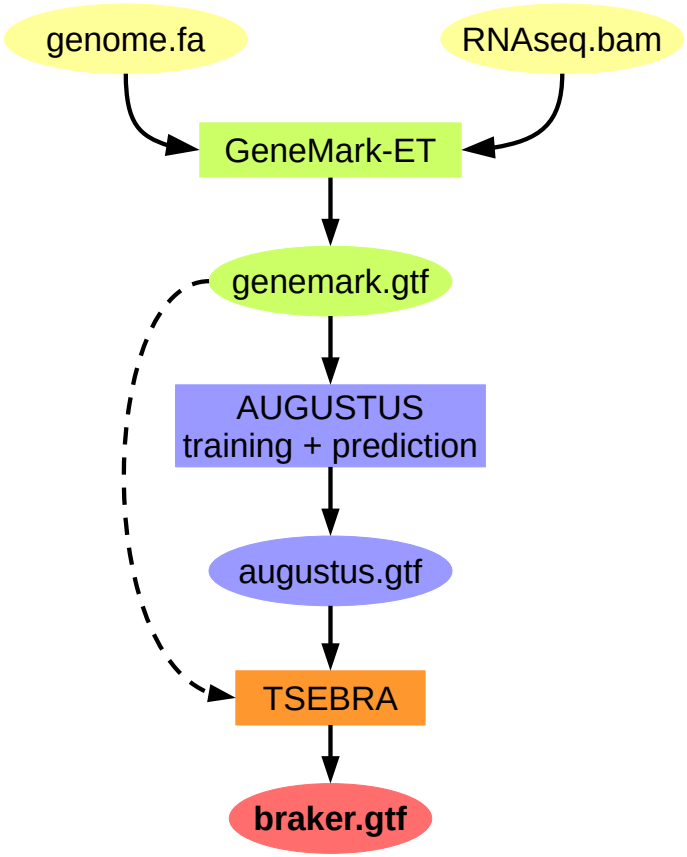
BRAKER1: Unsupervised RNA-Seq-Based Genome Annotation with GeneMark-ET and AUGUSTUS

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
- 1,218 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

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GeneMark-ET uses RNA-Seq for Training

Anchors from RNA-Seq for training

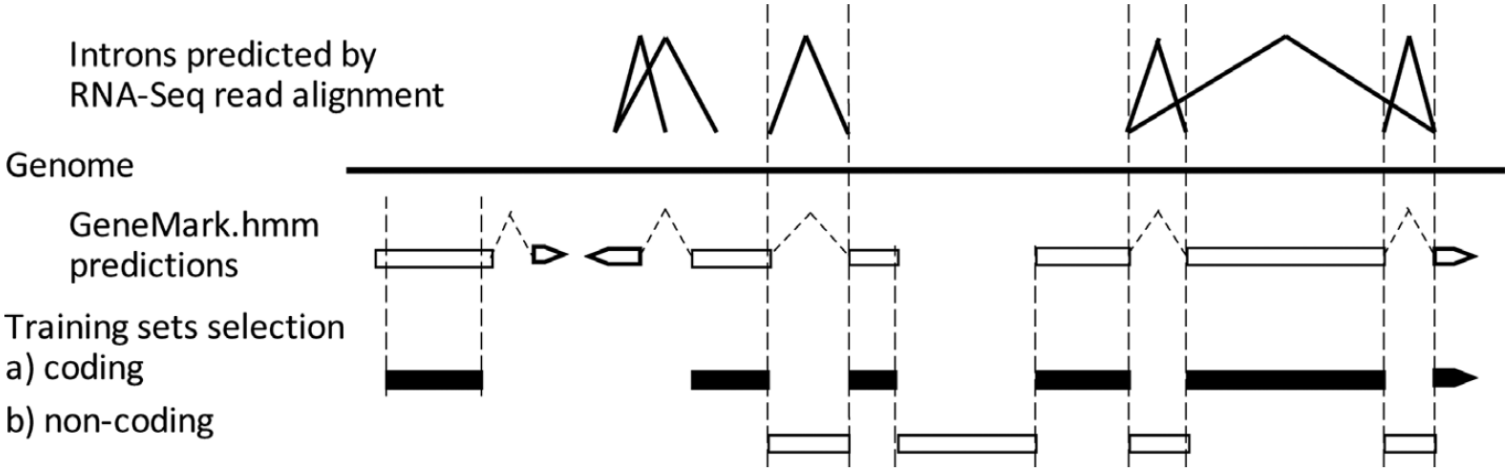

Figure 3. Selection of elements of training set in GeneMark-ET for the next iteration. The new training set of protein-coding regions is comprised from exons with at least one 'anchored splice site' as well as long exons predicted *ab initio* (>800 nt).

- employs unsupervised training
- includes in training 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

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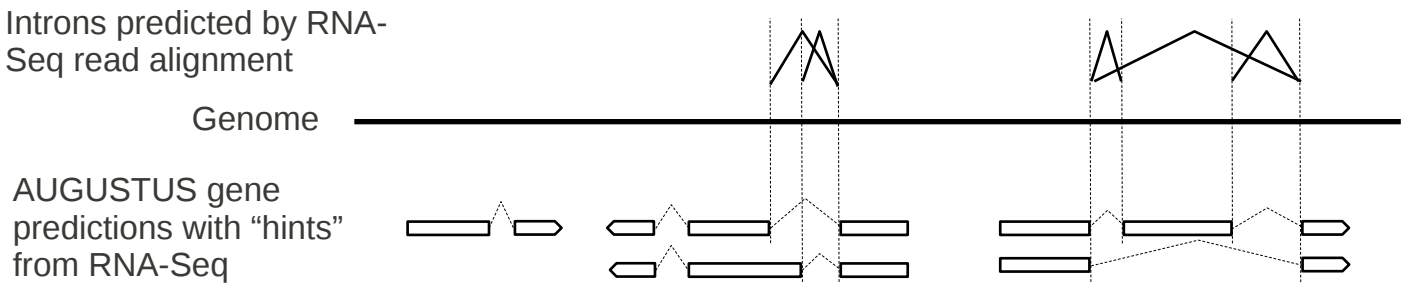
AUGUSTUS uses RNA-Seq for Prediction



- requires “prior data” for training
- uses intron information from RNA-seq for *prediction*
- no RNA-Seq assembly required

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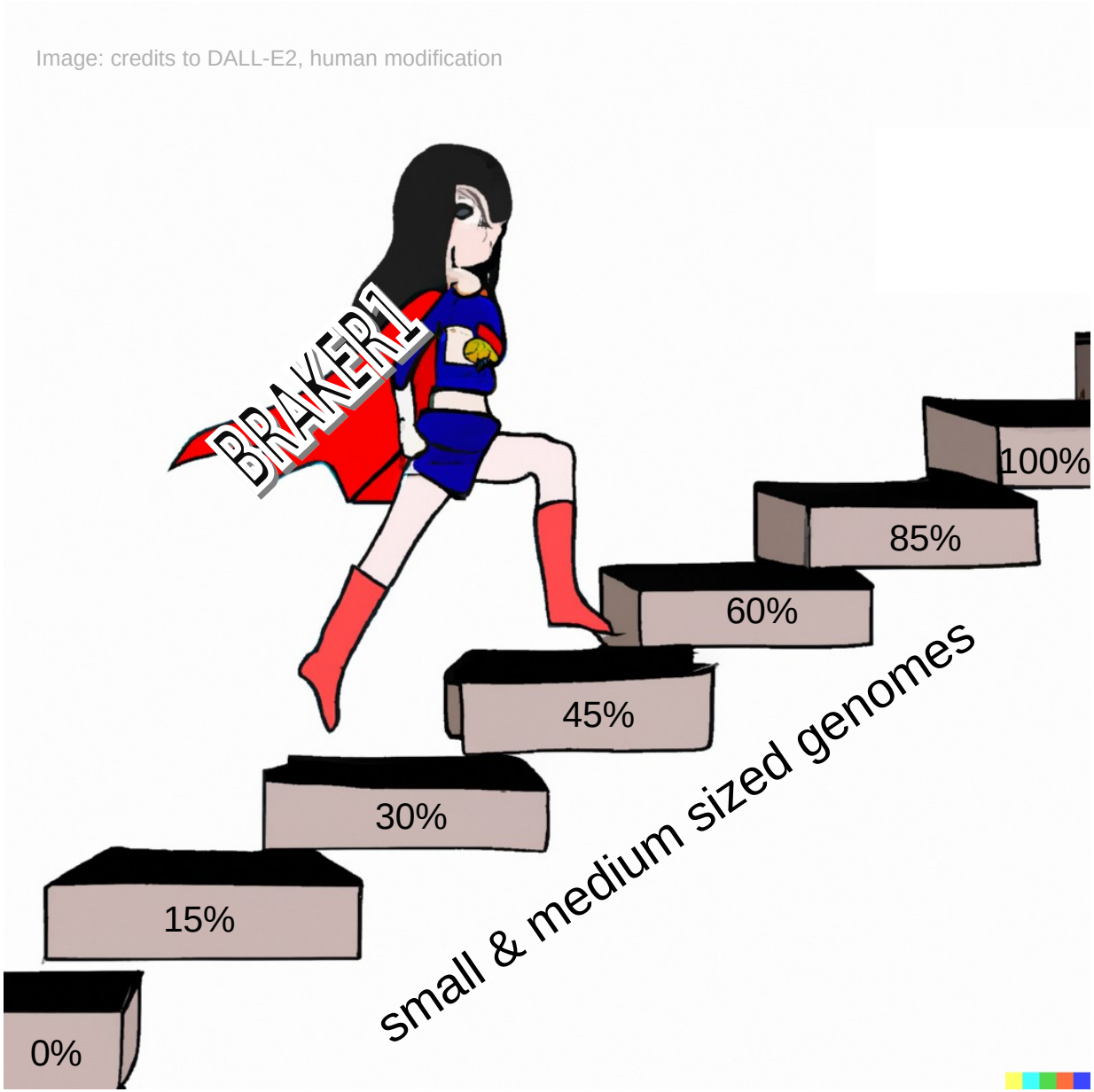
BRAKER1 Gene F1 Accuracy



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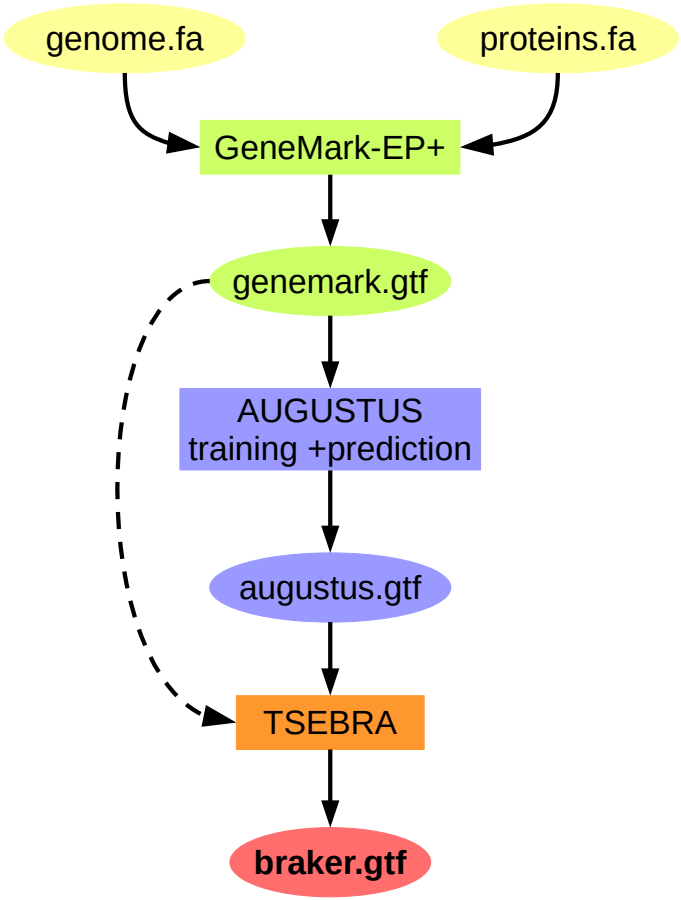
Published online 6 January 2021

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

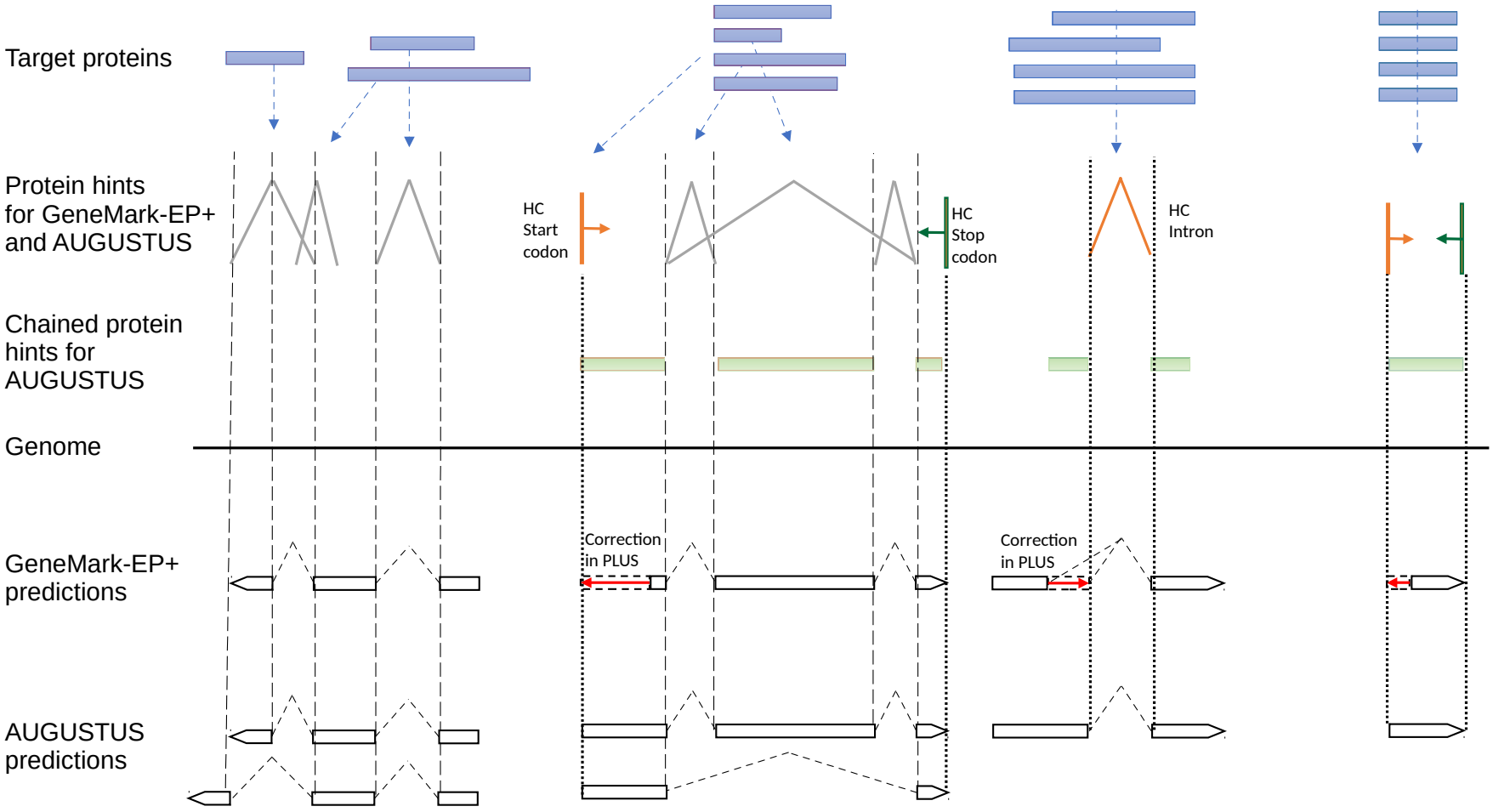
BRAKER2: automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS supported by a protein database

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
- 542 citations (Google Scholar)

Evidence Usage by GeneMark-EP+ & AUGUSTUS During Prediction



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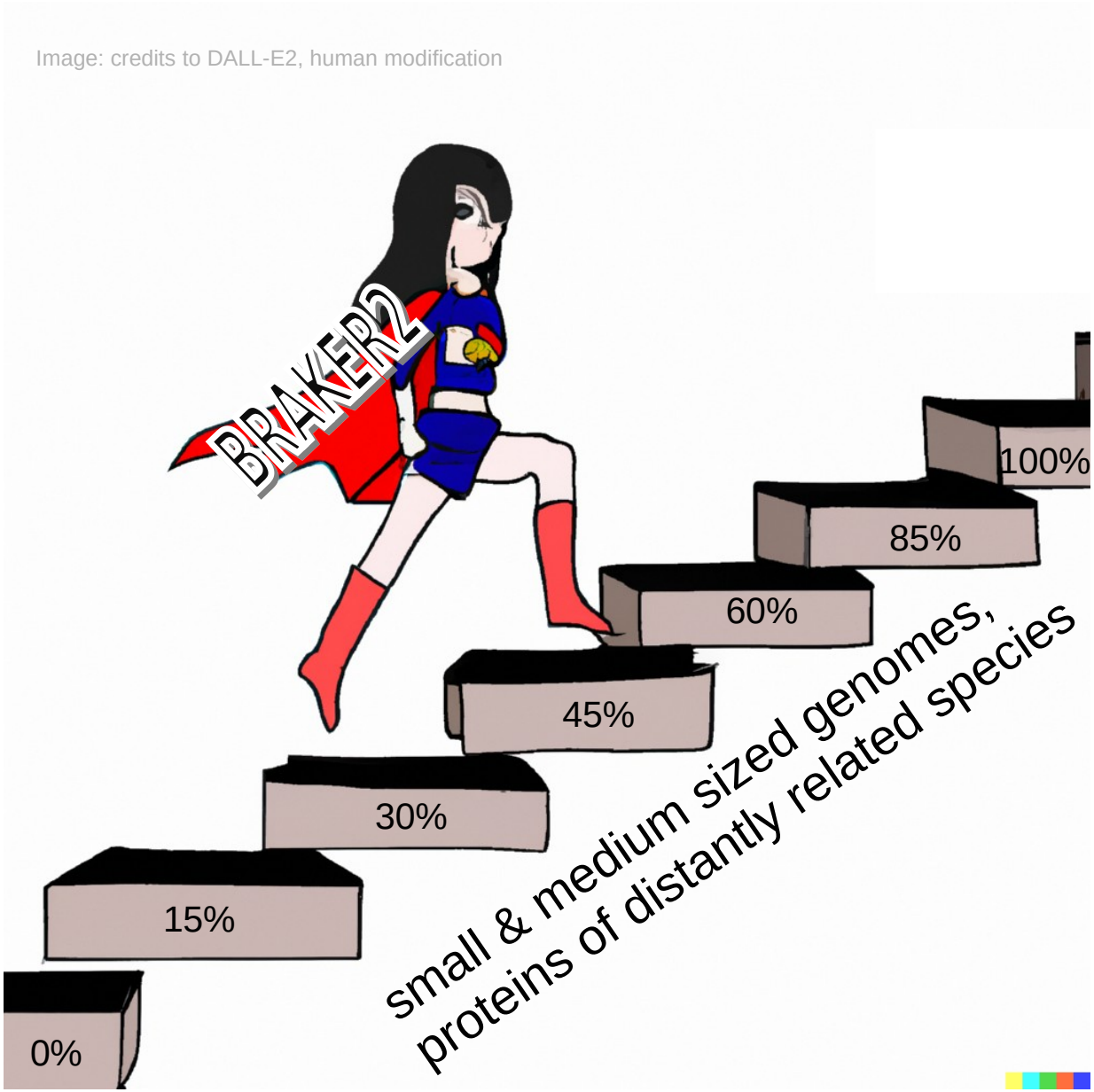
BRAKER2 Gene F1 Accuracy



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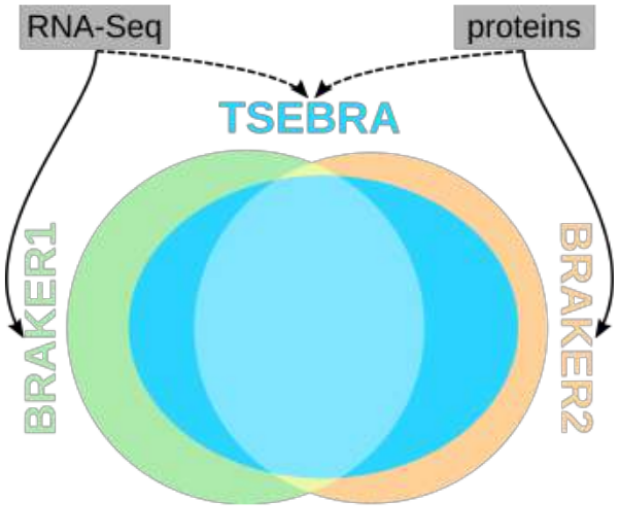
Gabriel *et al. BMC Bioinformatics* (2021) 22:566
https://doi.org/10.1186/s12859-021-04482-0

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*]



- run both BRAKER1 and BRAKER2
- combine & increase accuracy
- 45 citations (Google Scholar)

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BRAKER1: RNA-Seq

BRAKER2: Proteins

TSEBRA

BRAKER3: RNA-Seq + Proteins

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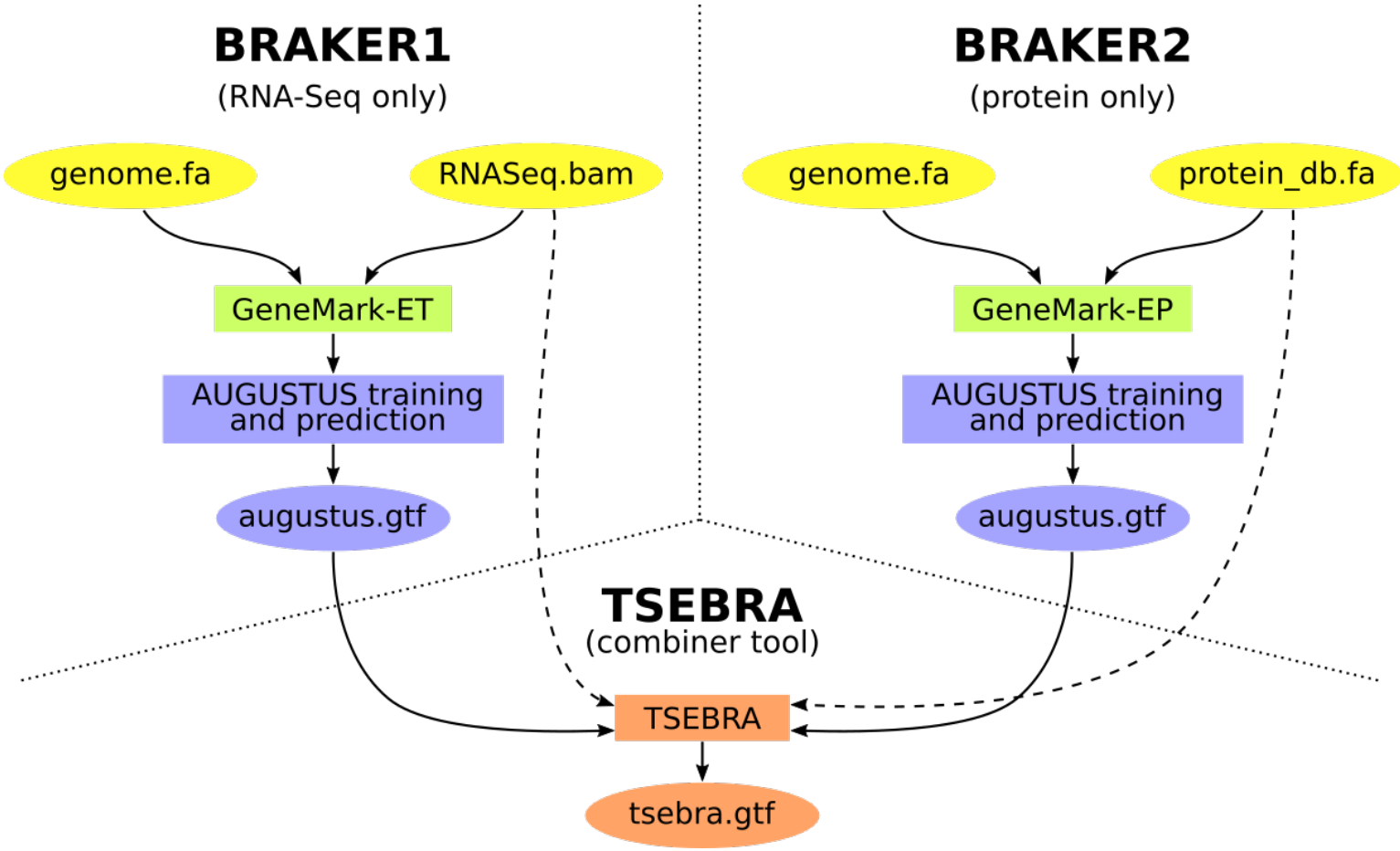
BRAKER1 + BRAKER2 → TSEBRA



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TSEBRA

BRAKER3: RNA-Seq + Proteins

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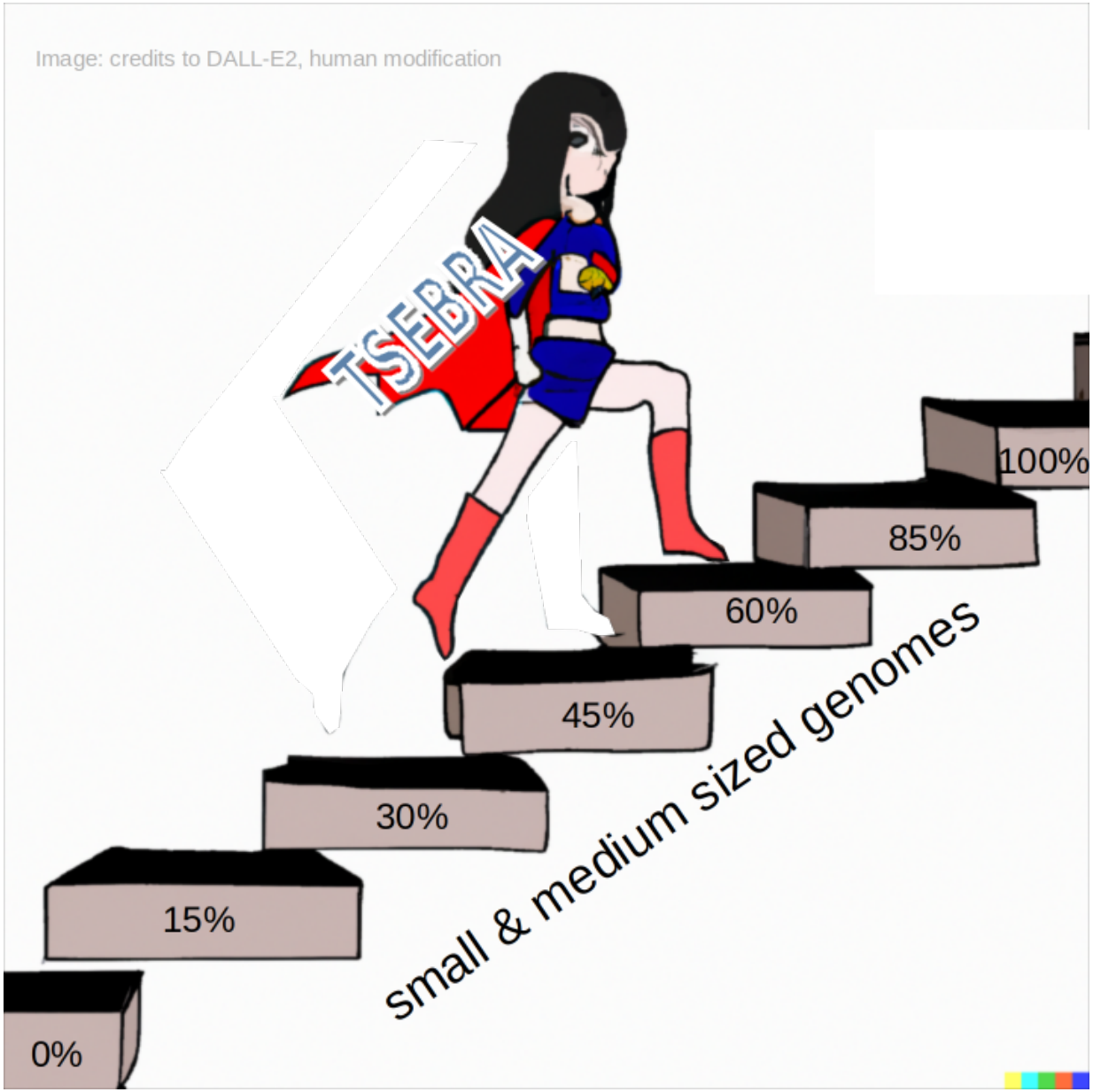
TSEBRA: BRAKER1 + BRAKER2 Gene F1 Accuracy



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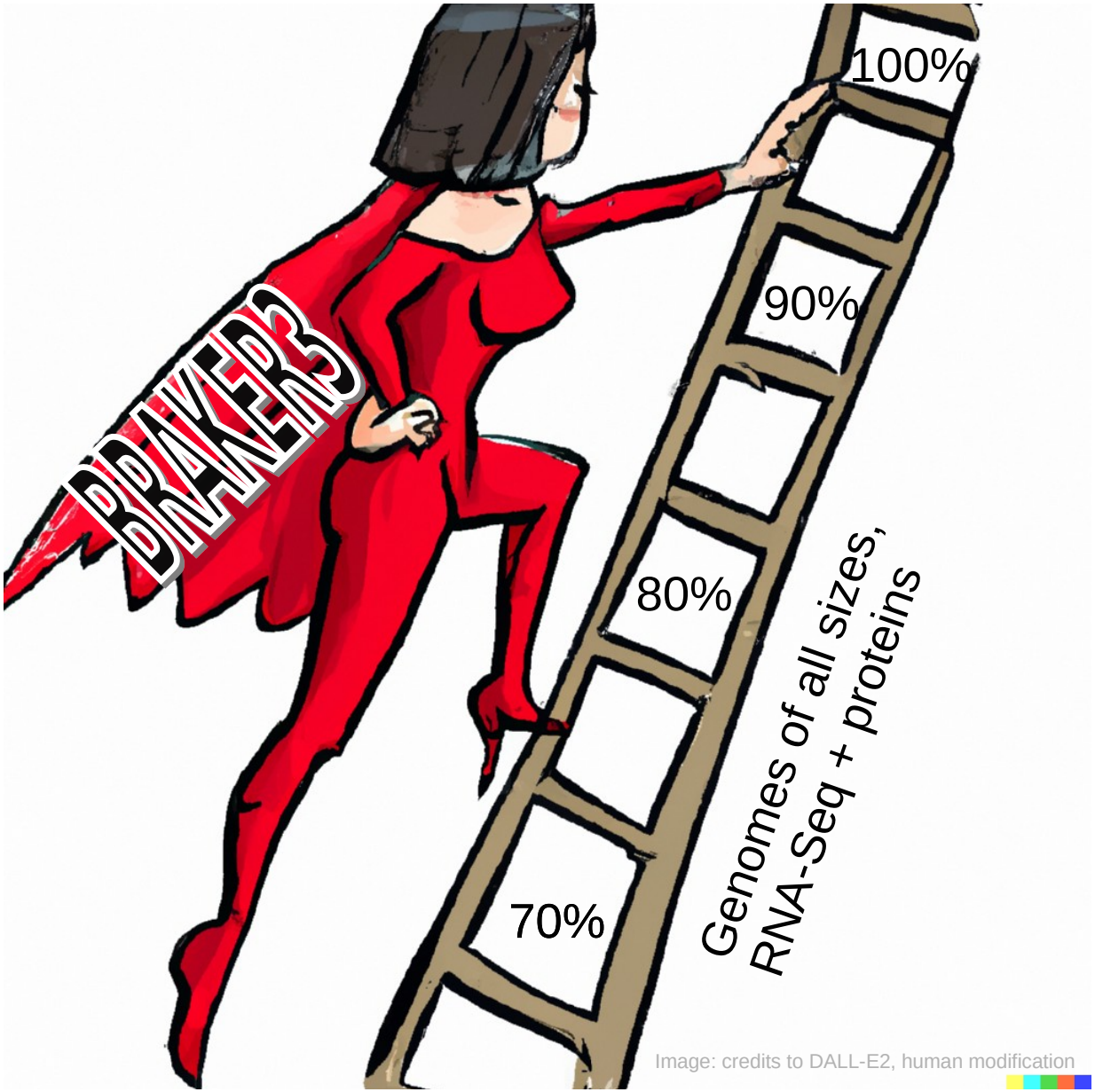
BRAKER3 Gene F1 Accuracy - Climbing the Top



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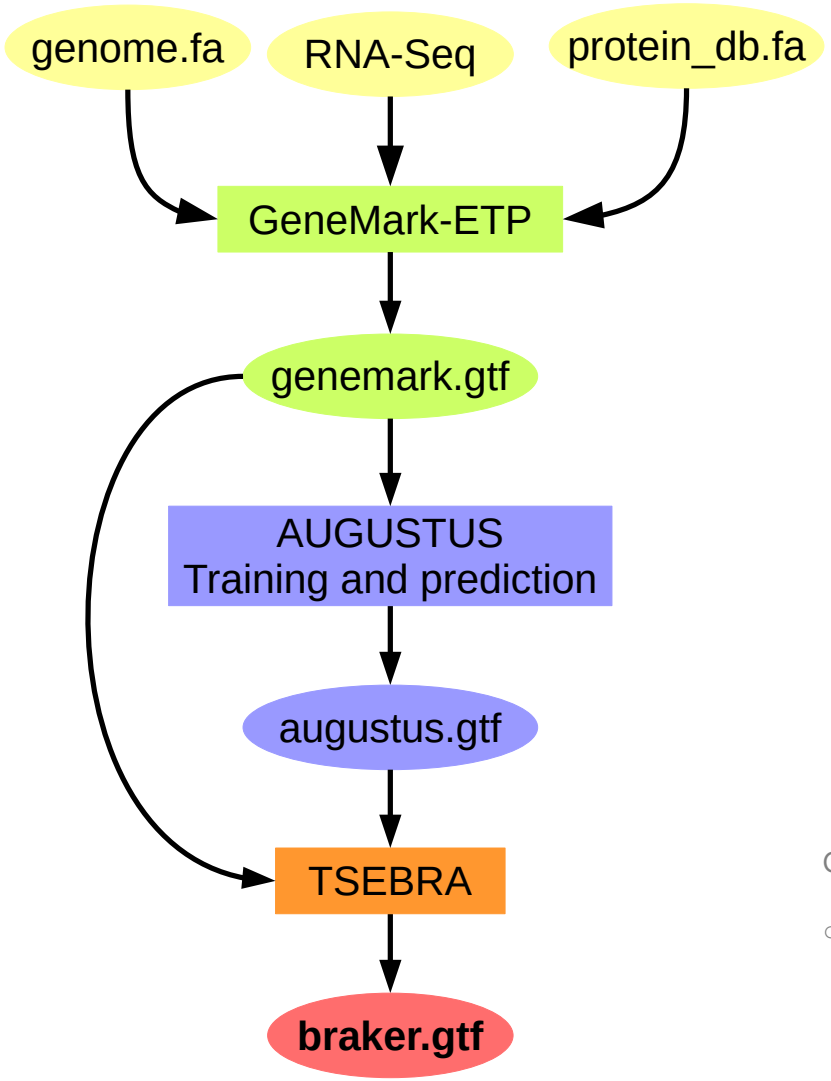
BRAKER3: Using RNA-Seq and Protein Evidence with GeneMark-ETP, AUGUSTUS and TSEBRA



- spliced aligned and **assembled** RNA-Seq
- large protein database
- combines GeneMark-ETP and AUGUSTUS gene sets with TSEBRA

Gabriel et al. (2023) https://www.biorxiv.org/content/10.1101/2023.06.10.544449v1

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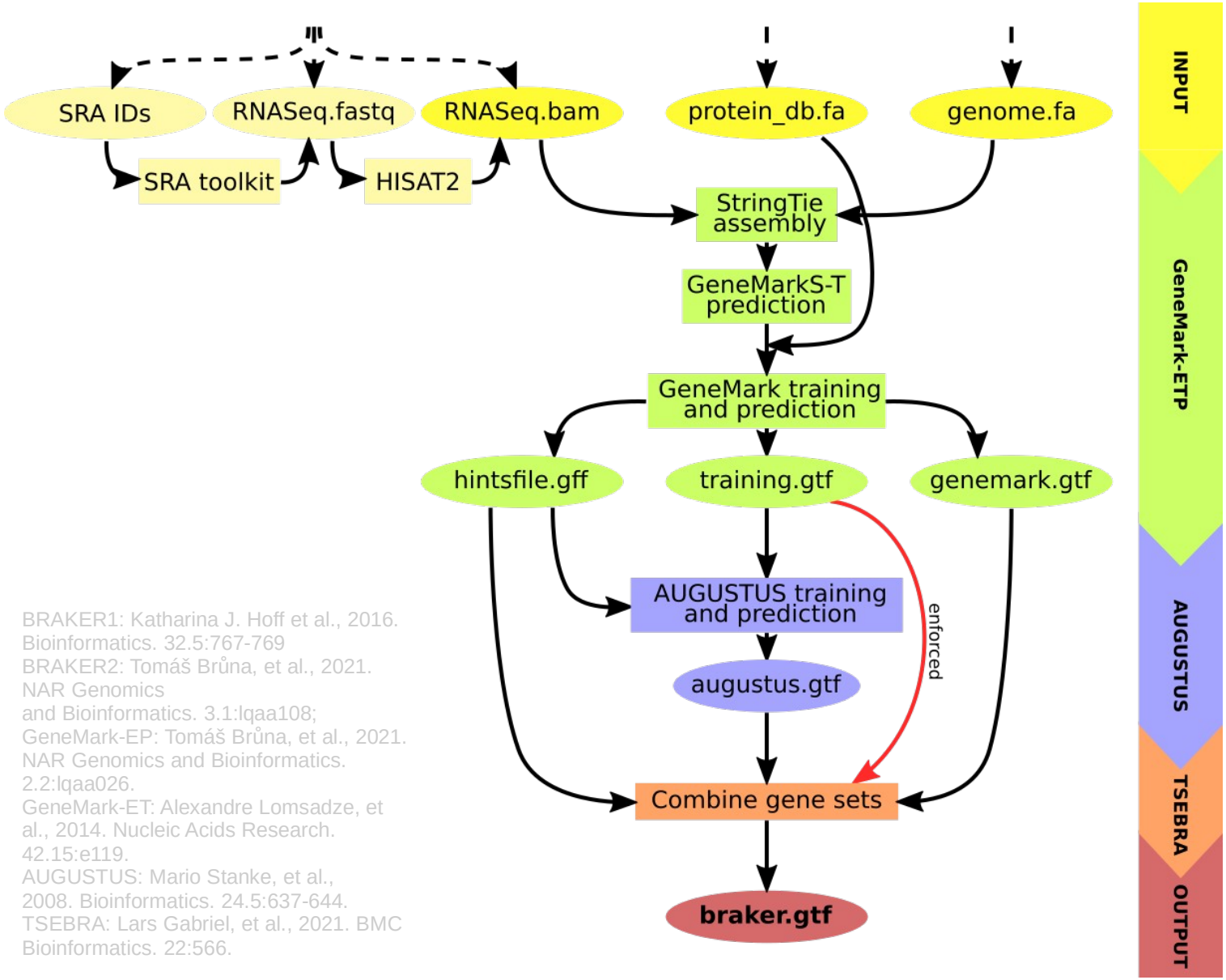

BRAKER1: Katharina J. Hoff et al., 2016. Bioinformatics. 32.5:767-769
BRAKER2: Tomáš Brůna, et al., 2021. NAR Genomics and Bioinformatics. 3.1:lqaa108;
GeneMark-EP: Tomáš Brůna, et al., 2021. NAR Genomics and Bioinformatics. 2.2:lqaa026.
GeneMark-ET: Alexandre Lomsadze, et al., 2014. Nucleic Acids Research. 42.15:e119.
AUGUSTUS: Mario Stanke, et al., 2008. Bioinformatics. 24.5:637-644.
TSEBRA: Lars Gabriel, et al., 2021. BMC Bioinformatics. 22:566.

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BRAKER3 Experiments

Experiments

Accuracy assessment using genome-wide predictions in 6 species:

Species	Genome Size (Mb)	# Genes in Annotation
Arabidopsis thaliana (thale cress)	119	27,444
Caenorhabditis elegans (nematode)	100	20,172
Drosophila melanogaster (fruit fly)	137	13,928
Gallus gallus (chicken)	1,040	17,279
Mus musculus (mouse)	2,650	22,378
Solanum lycopersicum (tomato)	772	33,562

Accuracy metrics

Specificity [Sp]: Percentage of correctly found genes/transcripts/exons in the **predicted gene set**.

Sensitivity [Sn]: Percentage of correctly found genes/transcripts/exons in the **reference annotation**.

Harmonic Mean [F1]: $$\frac{2 \cdot \text{Sensitivity} \cdot \text{Specificity}}{\text{Sensitivity} + \text{Specificity}}$$

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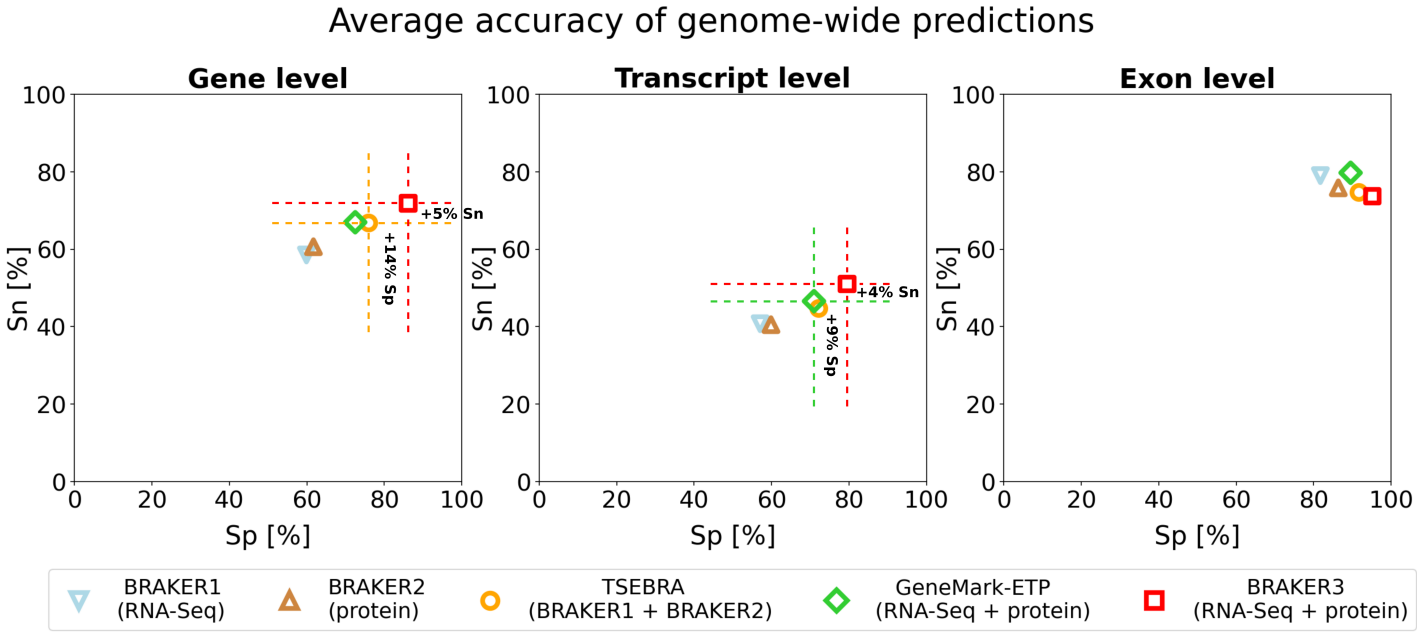
BRAKER3 Accuracy in Small Genomes



Species: *D. melanogaster*
A. thaliana
C. elegans

Extrinsic evidence:

- paired RNA-Seq short reads
- OrthoDB v.10 clade partitions **(order excluded)**

INPUT

GeneMark-ETP

AUGUSTUS

TSEBRA

OUTPUT

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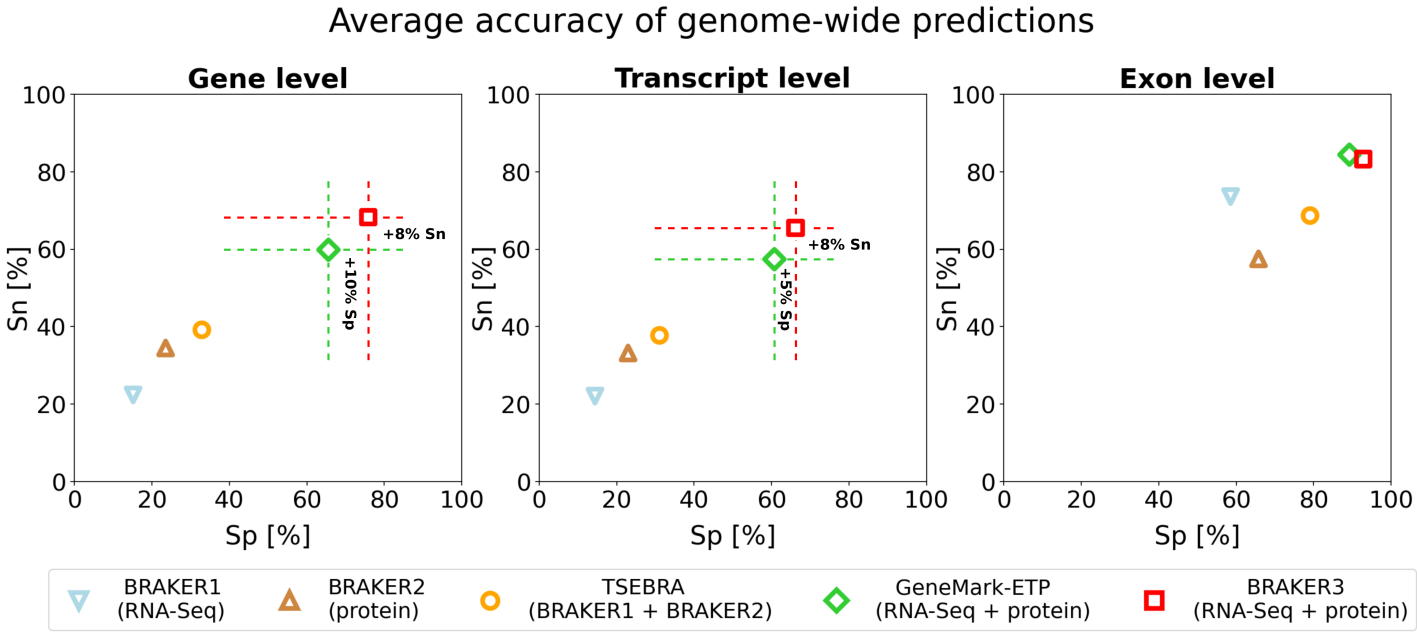
BRAKER3 Accuracy in Larger Genomes



Species: *M. musculus*
G. gallus
S. lycopersicum

Extrinsic evidence:

- paired RNA-Seq short reads
- OrthoDB v.10 clade partitions **(order excluded)**

INPUT
GeneMark-ETP
AUGUSTUS
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Usage & Runtime

Command line

```
braker.pl --genome=genome.fa --prot_seq=protein_db.fa \
          --rnaseq_sets_ids=RNA_ID1,RNA_ID2 \
          --rnaseq_sets_dirs=/path/to/RNASeq/
```

Runtime

- average for *A. thaliana*, *C. elegans*, *D. melanogaster*, *G. gallus*, *M. musculus*, *S. lycopersicum*.
- with 48 threads:

	BRAKER1	BRAKER2	GM-ETP	BRAKER3
Runtime (h)	06:26	09:01	06:03	17:55

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Availability

GitHub

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

Docker/Singularity

```
singularity build braker3.sif \
            docker://teambraker/braker3:latest

singularity exec braker3.sif braker.pl [OPTIONS]
```

Licenses

- BRAKER: Artistic License
- GeneMark-ETP: License for GeneMark family software `http://topaz.gatech.edu/genemark/license_download.cgi`

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The GALBA Team

DOE Joint Genome Institute & Harvard Medical School & University of Greifswald

Tomáš Brůna

Heng Li

Lars Gabriel

Natalia Nenasheva

Matthis Ebel

Mario Stanke

Katharina Hoff

Also: Joseph Guhlin, Daniel Honsel, & Steffen Herboldt

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Miniprot

Bioinformatics, 39(1), 2023, btad014
https://doi.org/10.1093/bioinformatics/btad014
Advance Access Publication Date: 17 January 2023
Original Paper

OXFORD

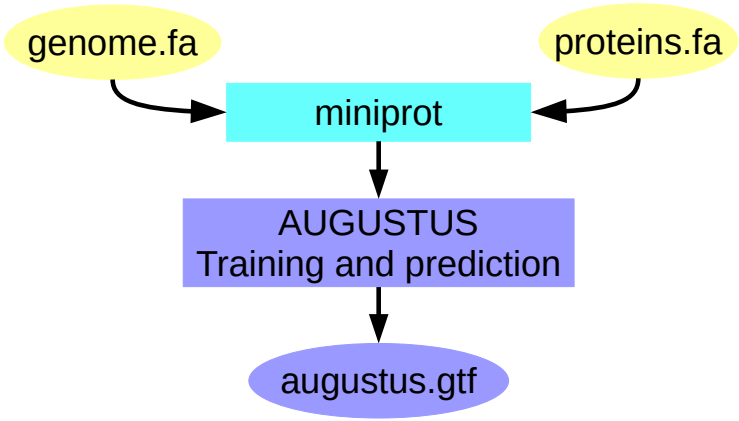
Genome analysis

Protein-to-genome alignment with miniprot

Heng Li [1,2]

"*Miniprot is a fast protein-to-genome aligner comparable to existing tools in accuracy. Its primary use case is to assist gene annotation.*"

GALBA



Do we need another pipeline?

- no RNA-Seq, large genome
- proteins of few or 1 reference species
- fully open source

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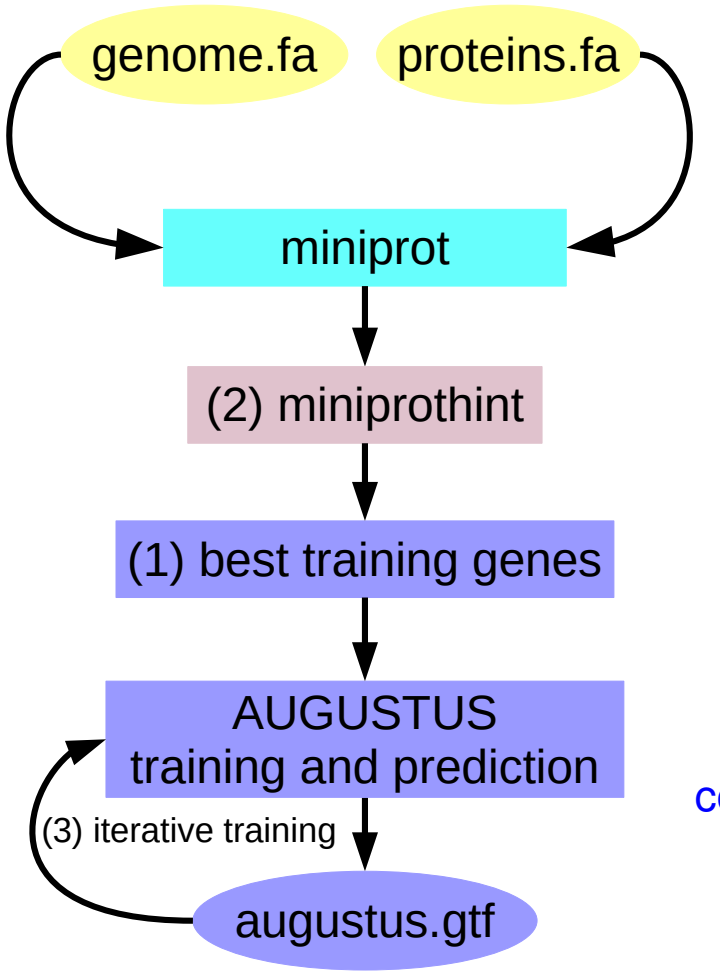
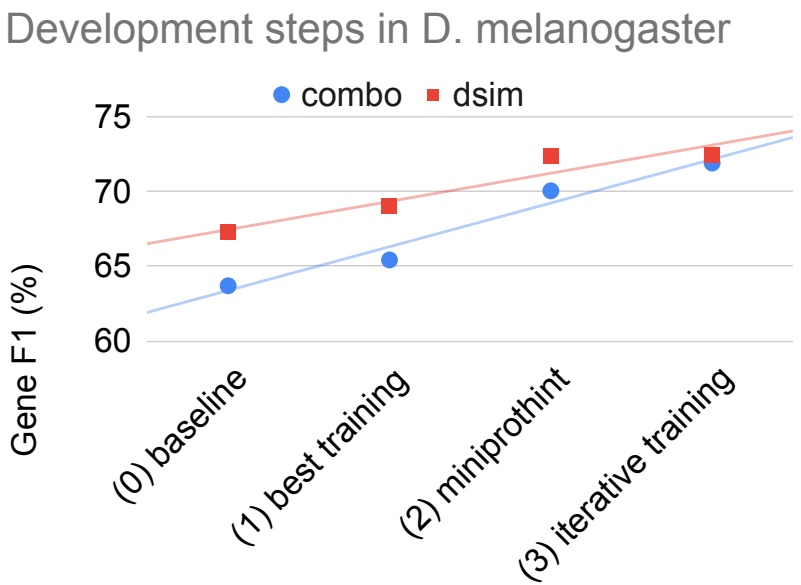
GALBA: Proteins of Closely Related Species





Donor proteins from

dsim *D. simulans*

combo *D. ananassae*, *D. pseudoobscura*, *D. willistoni*, *D. virilis*, *D. grimshawi*

Bruna *et al.* (2023) https://doi.org/10.1186/s12859-023-05449-z

Introduction

BRAKER

BRAKER1: RNA-Seq

BRAKER2: Proteins

TSEBRA

BRAKER3: RNA-Seq + Proteins

Accuracy Results

Availability

GALBA

GALBA: Proteins

Accuracy Results

Availability

Annotation Quality

Genome Browsers

Descriptive Statistics

BUSCO

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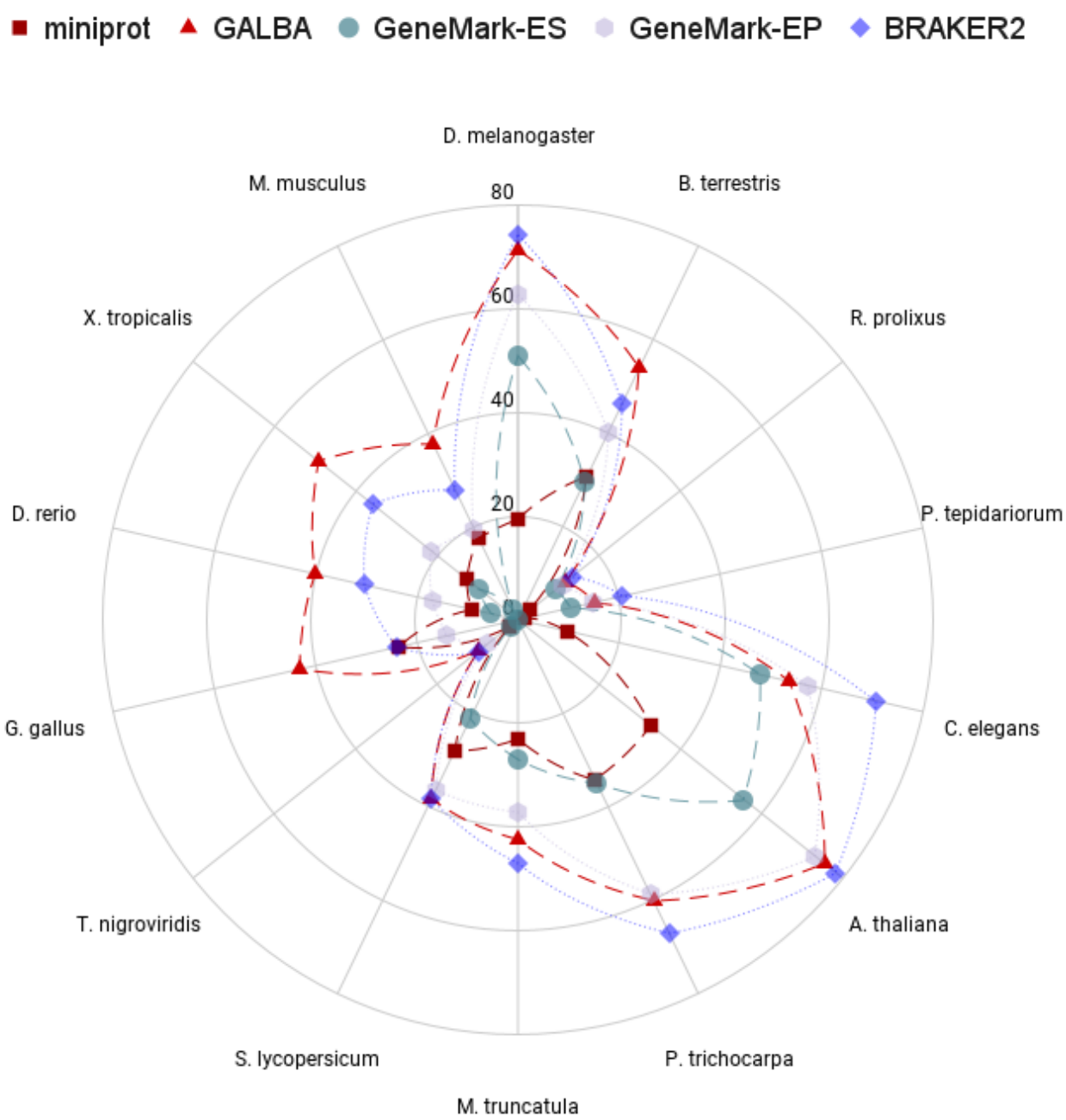
Gene F1 of GALBA in 14 Species



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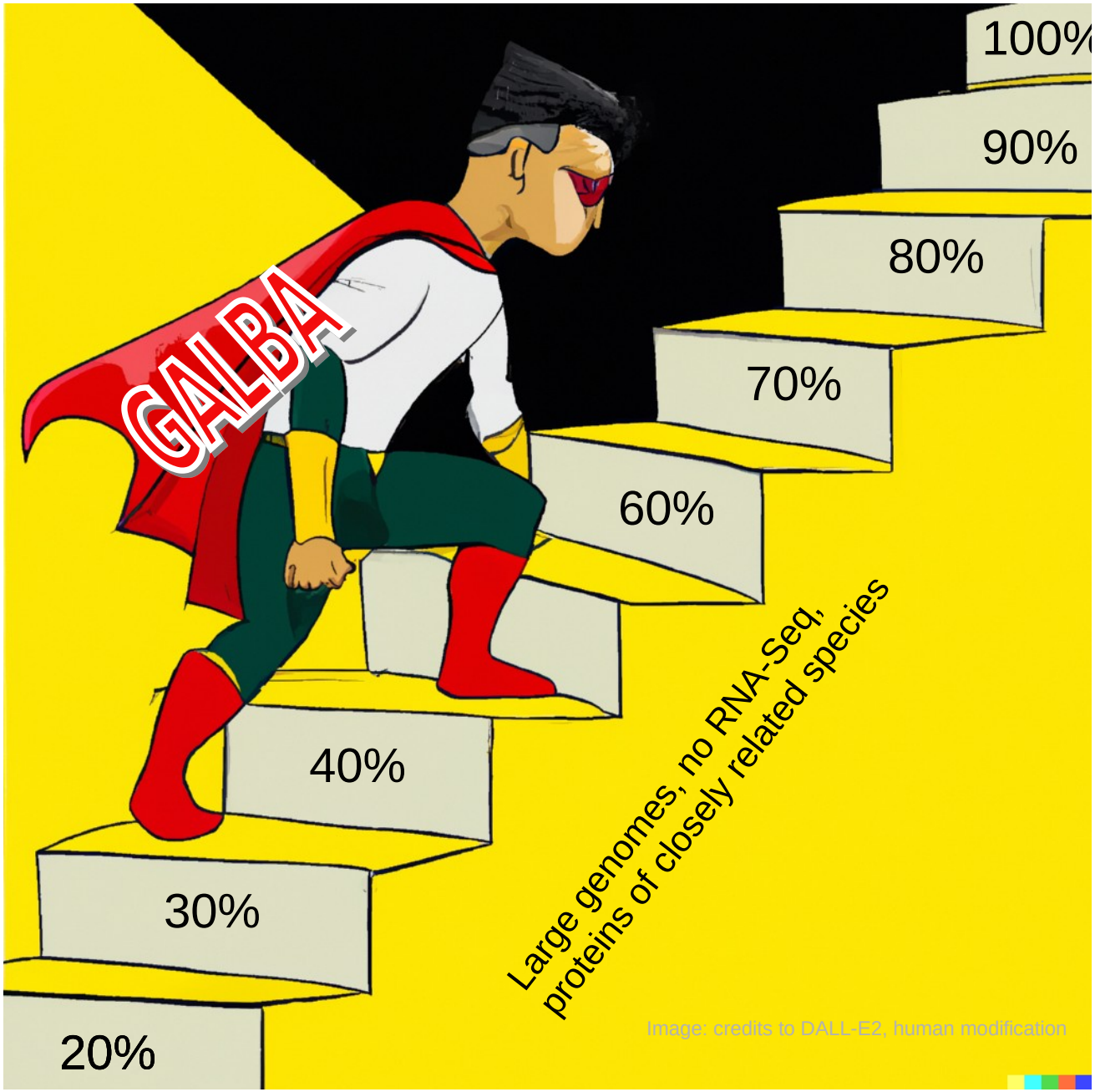
GALBA: Gene F1 Accuracy



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Did We Do a Good Job?

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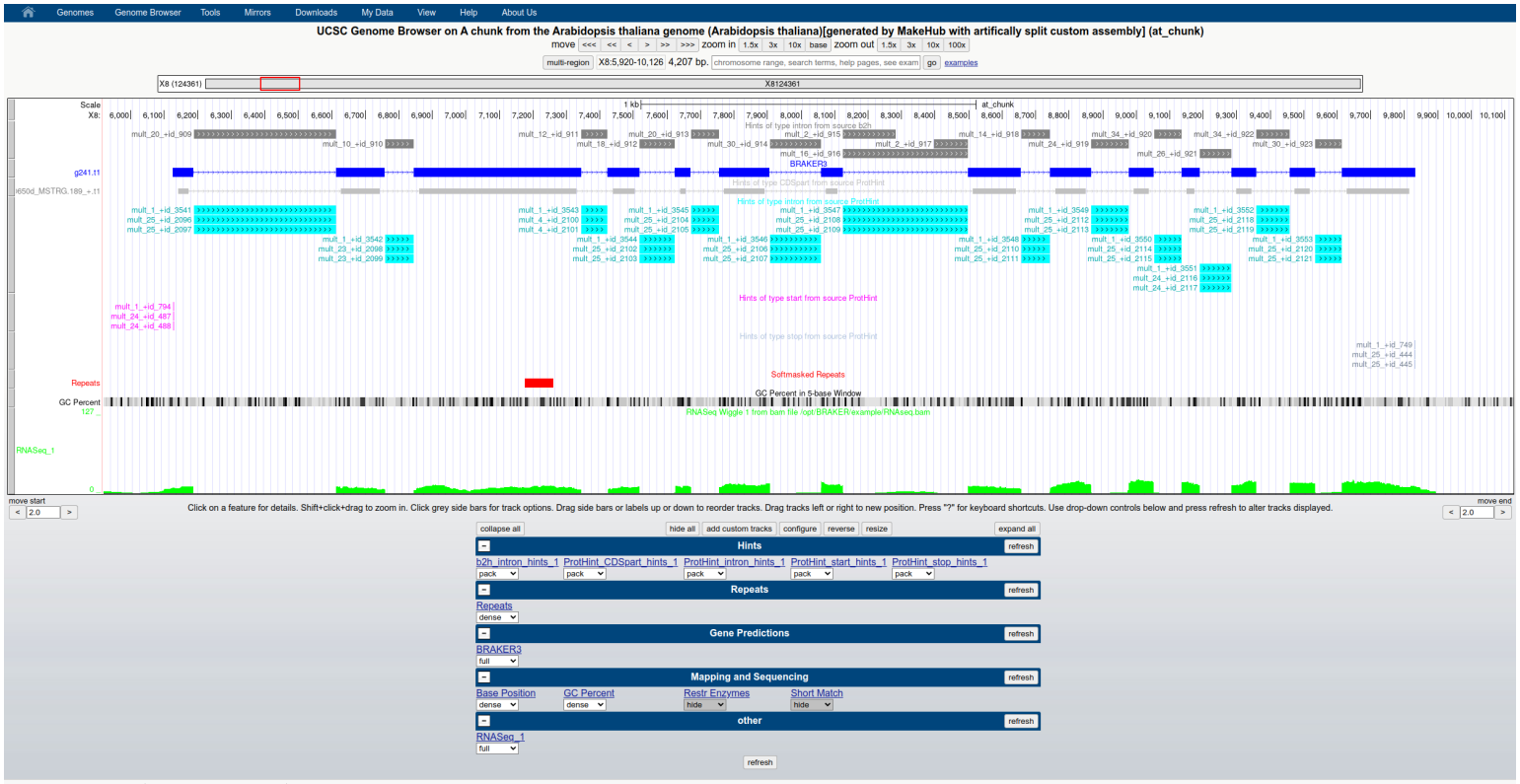
Genome Browsers

Visualize your Annotation in Context with Evidence

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



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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.

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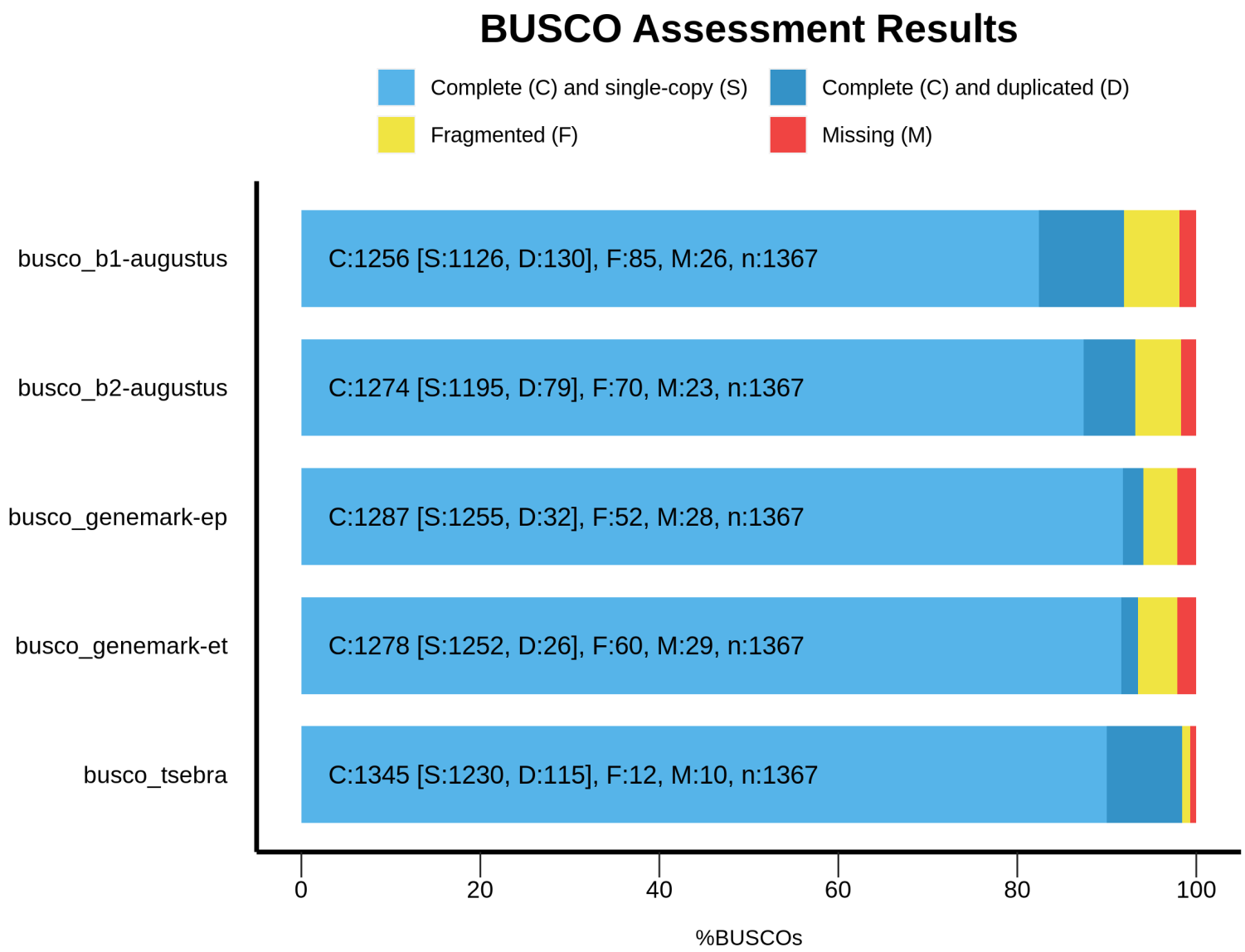
BUSCO: Sensitivity in Clade-Specific Conserved Genes



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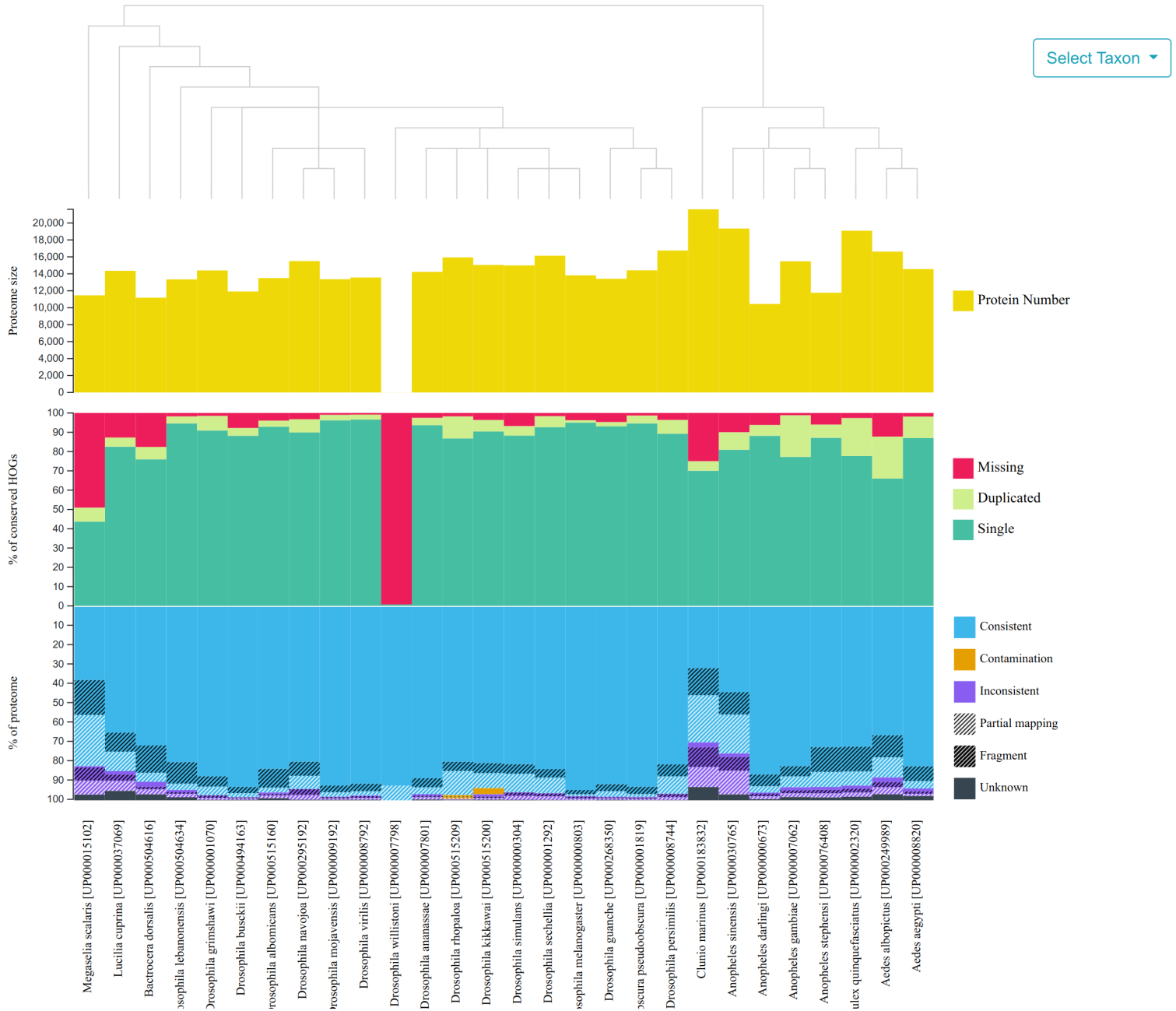
OMArk: Sensitivity, Contaminations, & More



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Organization of Session

1. start GitPod with the B3 repository
2. open the file tutorial.md
3. we walk you through that file
4. copy & paste the BRAKER3 command into the terminal and execute
5. (runtime is 30 minutes with 8 threads)
6. we will inspect a pre-computed results folder
7. time for your questions and discussion

Getting Started

Go to `https://gitpod.io/#https://github.com/BGAcademy23/B3`

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Funding

BRAKER

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GALBA

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