Annotation of Protein Coding Genes

January 16th 2026

Katharina J. Hoff

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Mastodon: @KatharinaHoff@fosstodon.org

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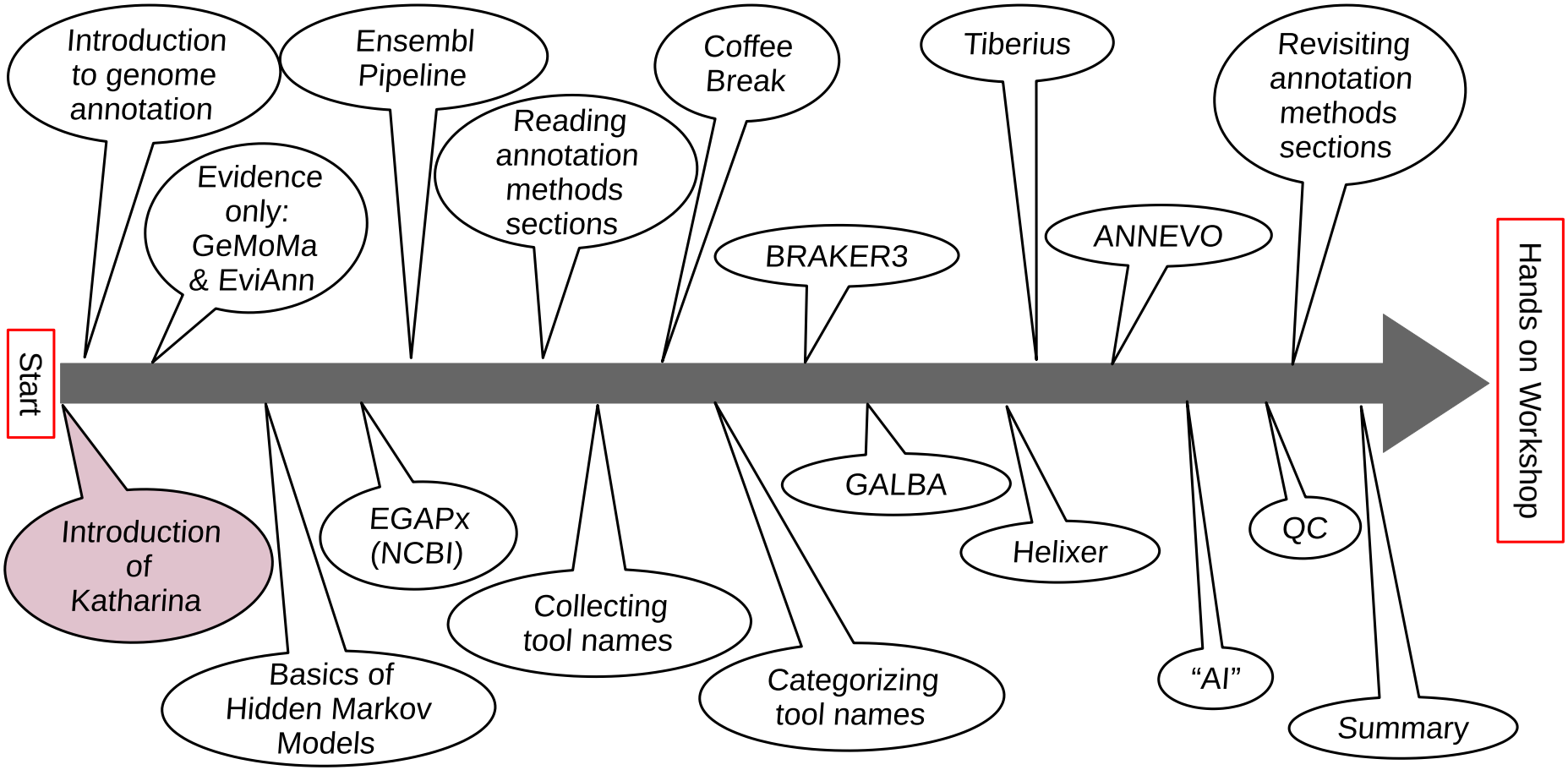

Introduction to genome annotation
Ensembl Pipeline
Coffee Break
Tiberius
Revisiting annotation methods sections
Evidence only: GeMoMa & EviAnn
Reading annotation methods sections
BRAKER3
ANNEVO
Start
Hands on Workshop
Introduction of Katharina
EGAPx (NCBI)
GALBA
QC
Helixer
Collecting tool names
Basics of Hidden Markov Models
Categorizing tool names
“AI”
Summary

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)

2025 DFG Heisenberg grant → professor in 2026?

Research

- eukaryotic genome annotation, metagenomics
- best known for: **BRAKER** & other **Gaius-Augustus** software
- 41 peer-reviewed research articles with currently 9,357 citations
- ∼1.9 Mio € grants

Teaching

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

... I love to sail, have 2 dogs, a cat, and an 9-years old daughter...

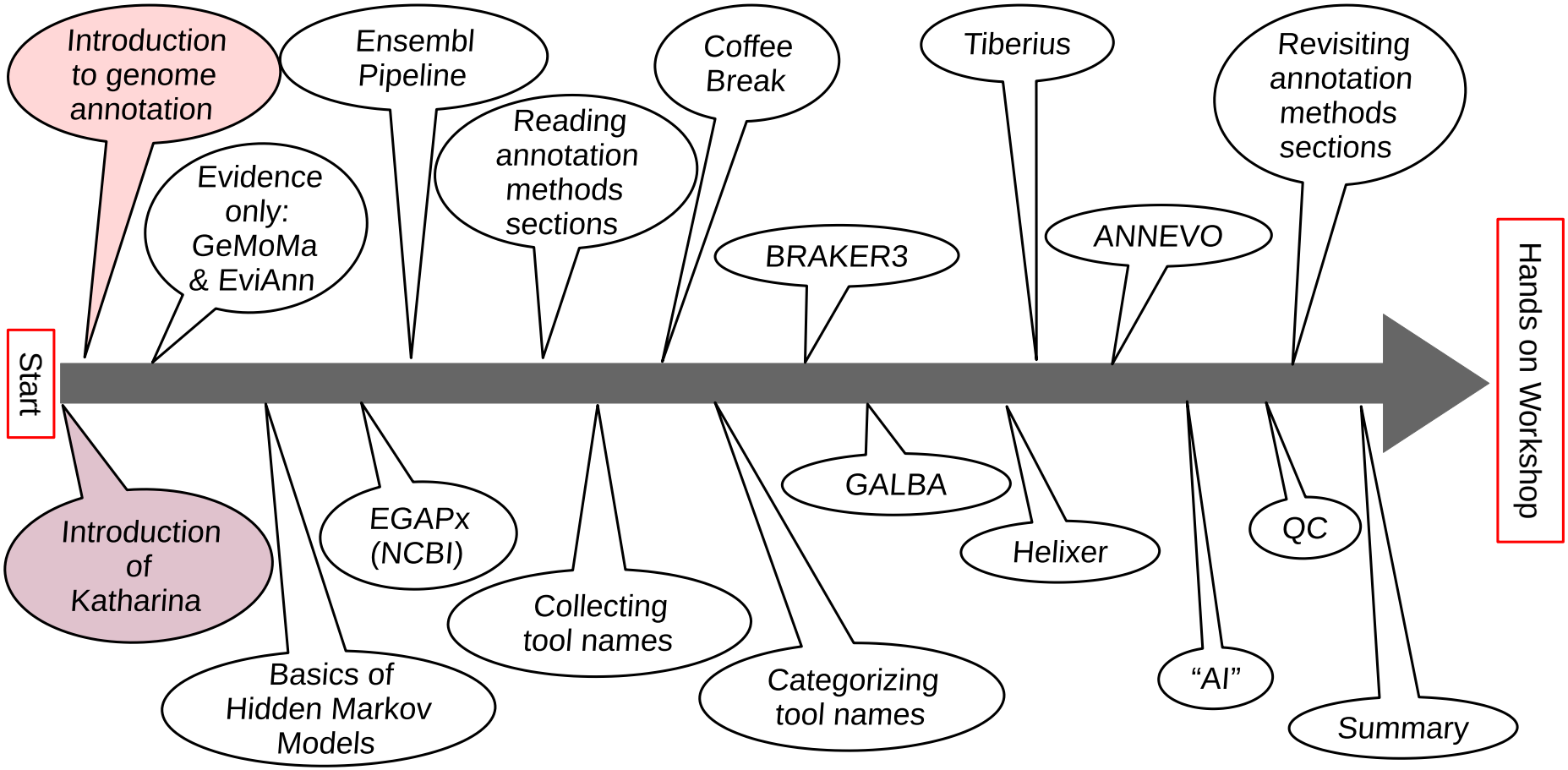
After this lecture, you will...

- 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.)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Where are the protein coding genes?

Genomic sequence: chicken

cctcacctctgagaaaacctctttgccaccaataccatgaagctctgcgtgactgtcctgtctctcctc
gtgctagtagctgccttctgctctctagcactctcagcaccaagtaagtctacttttgcagctgctatt
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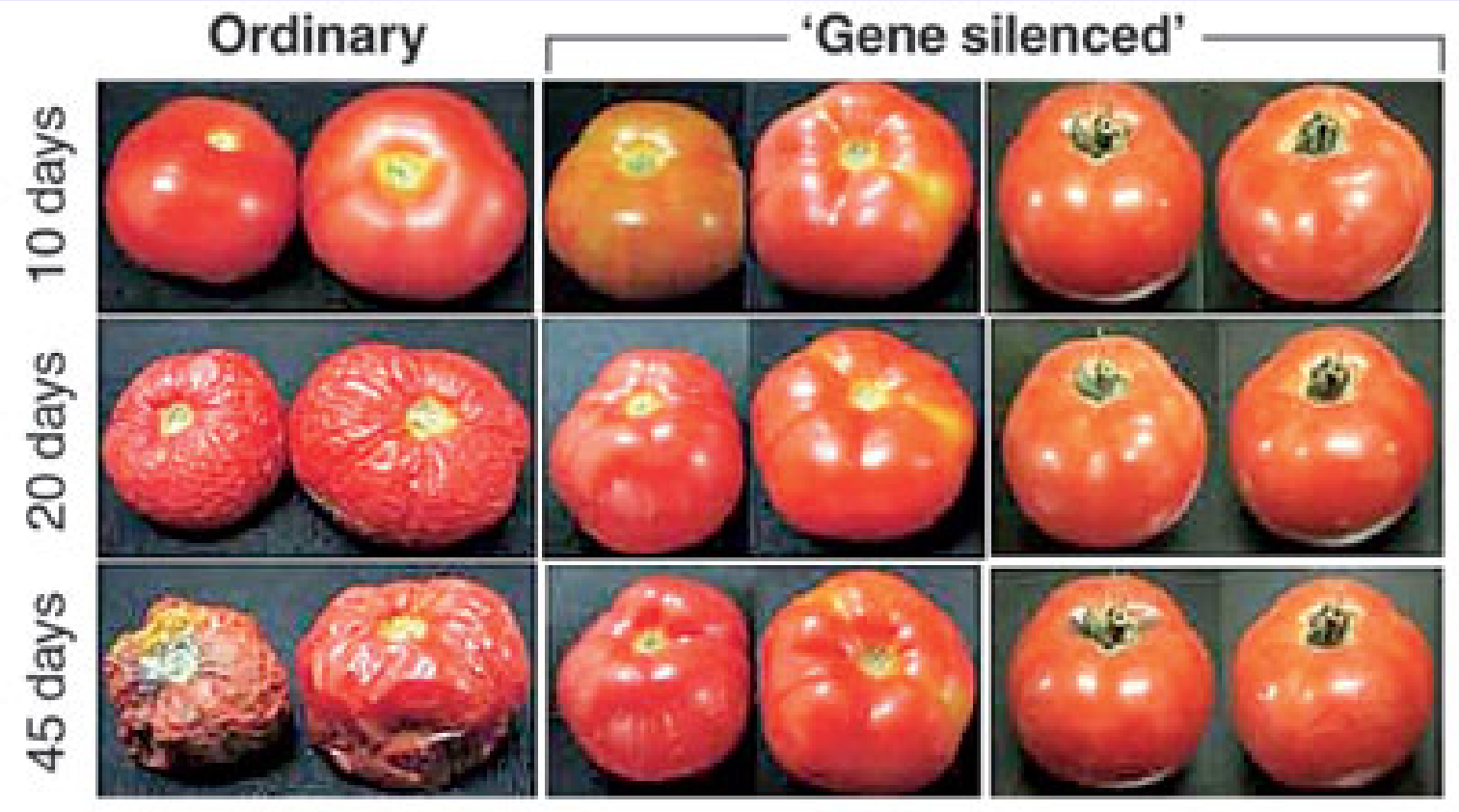
Examples for the importance of genome annotation

Silencing polygalacturonase activity in tomato



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

Bacillus thuringiensis toxin against European corn borer

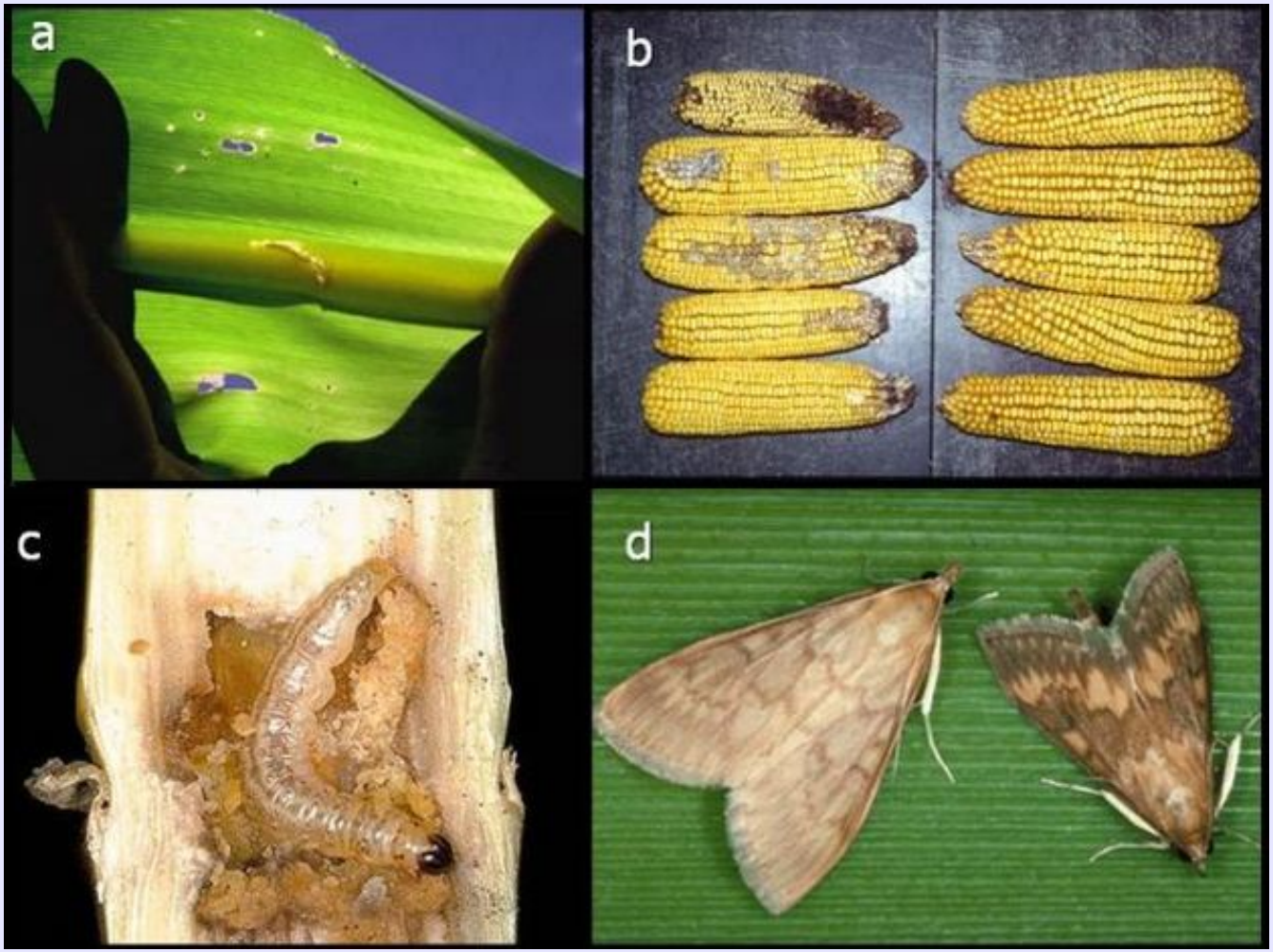


Image: Hellmich & Hellmich (2012) Nature Education Knowledge 3(10):4

`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**

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  - European harvest mouse: O'Brien & Colom (2024), **2 authors**
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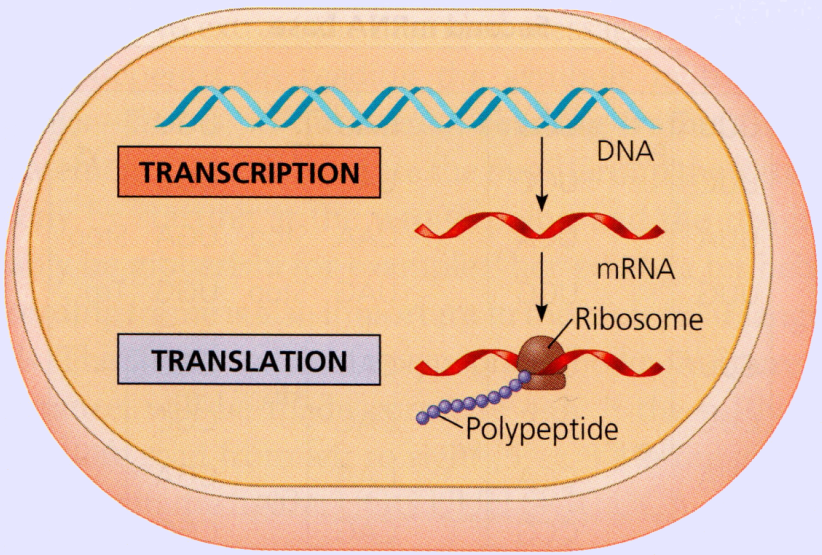
- **You can do it!**

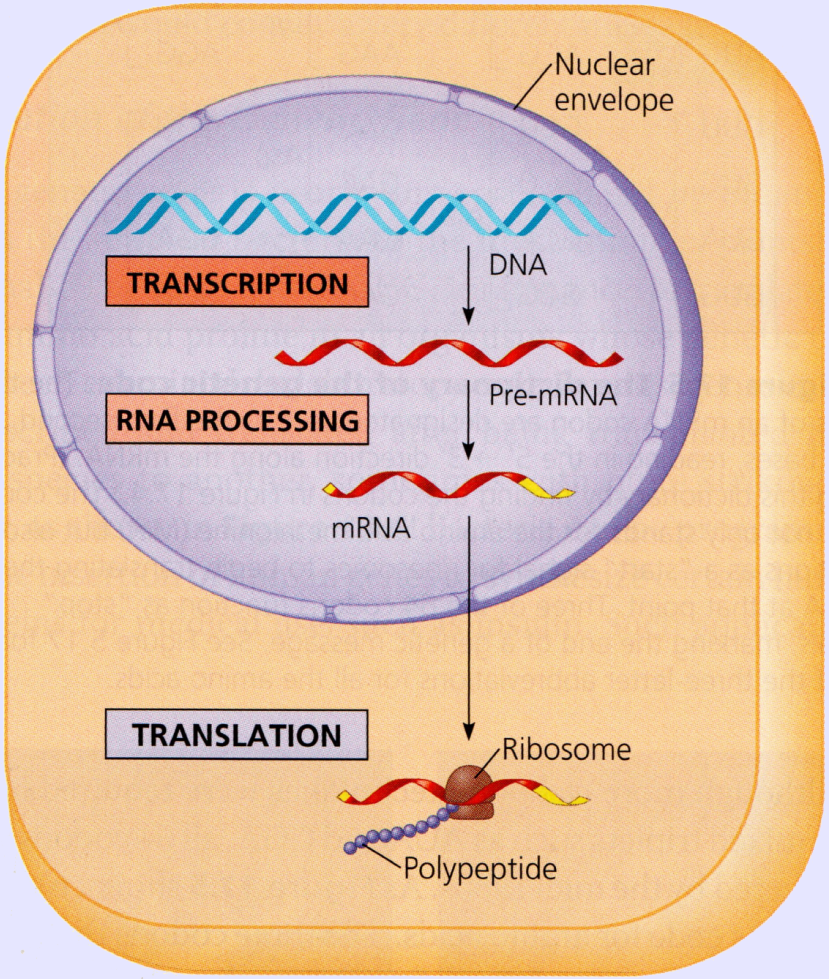
How does a cell recognize protein-coding genes?

Transcription & Translation

Prokaryotes



Eukaryotes



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

How does a cell recognize protein-coding genes?

Prokaryotes & Eukaryotes*

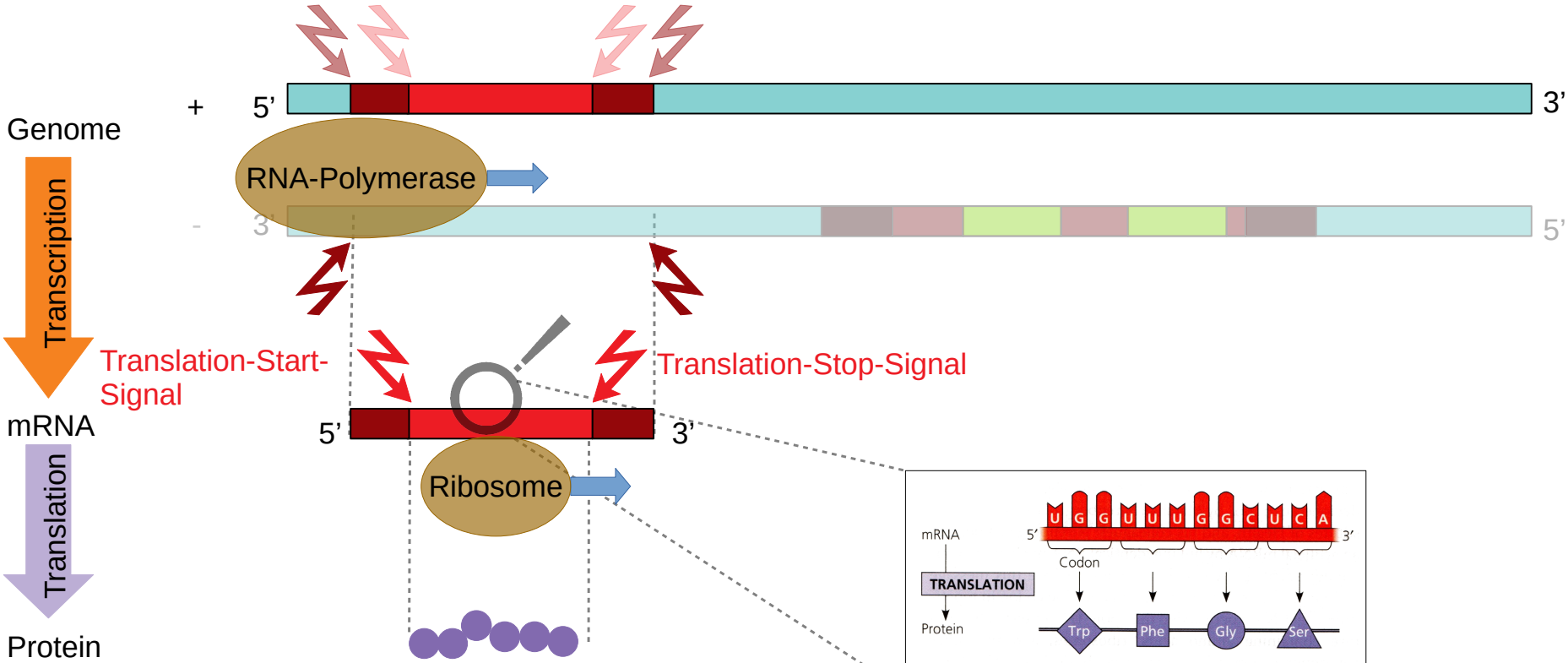


Image: Campbell et al., Biology, San Francisco, 2008, p. 329, FIg. 17.4

*) only some of the genes in eukaryotes

How does a cell recognize protein-coding genes?

Prokaryotes & Eukaryotes*

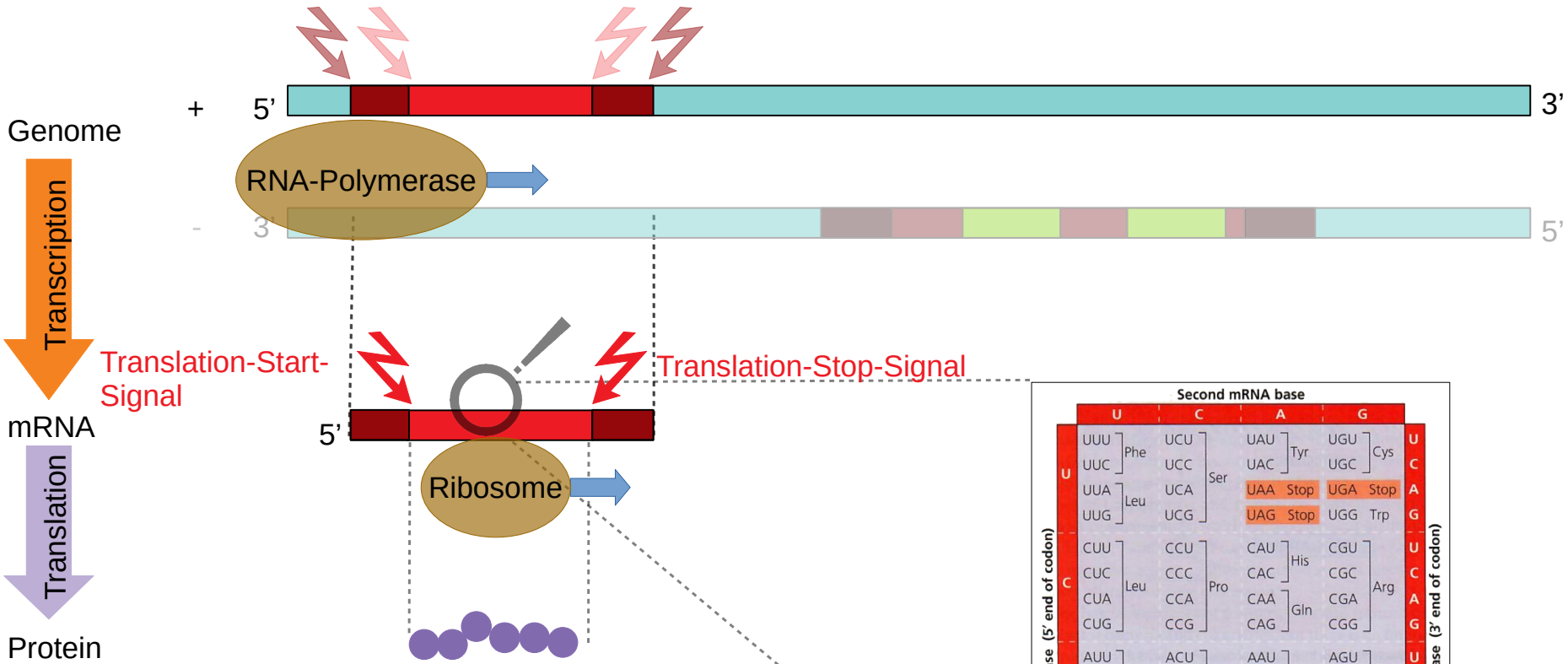


Image: Campbell et al., Biology, San Francisco, 2008, p. 339, Fig. 17.5

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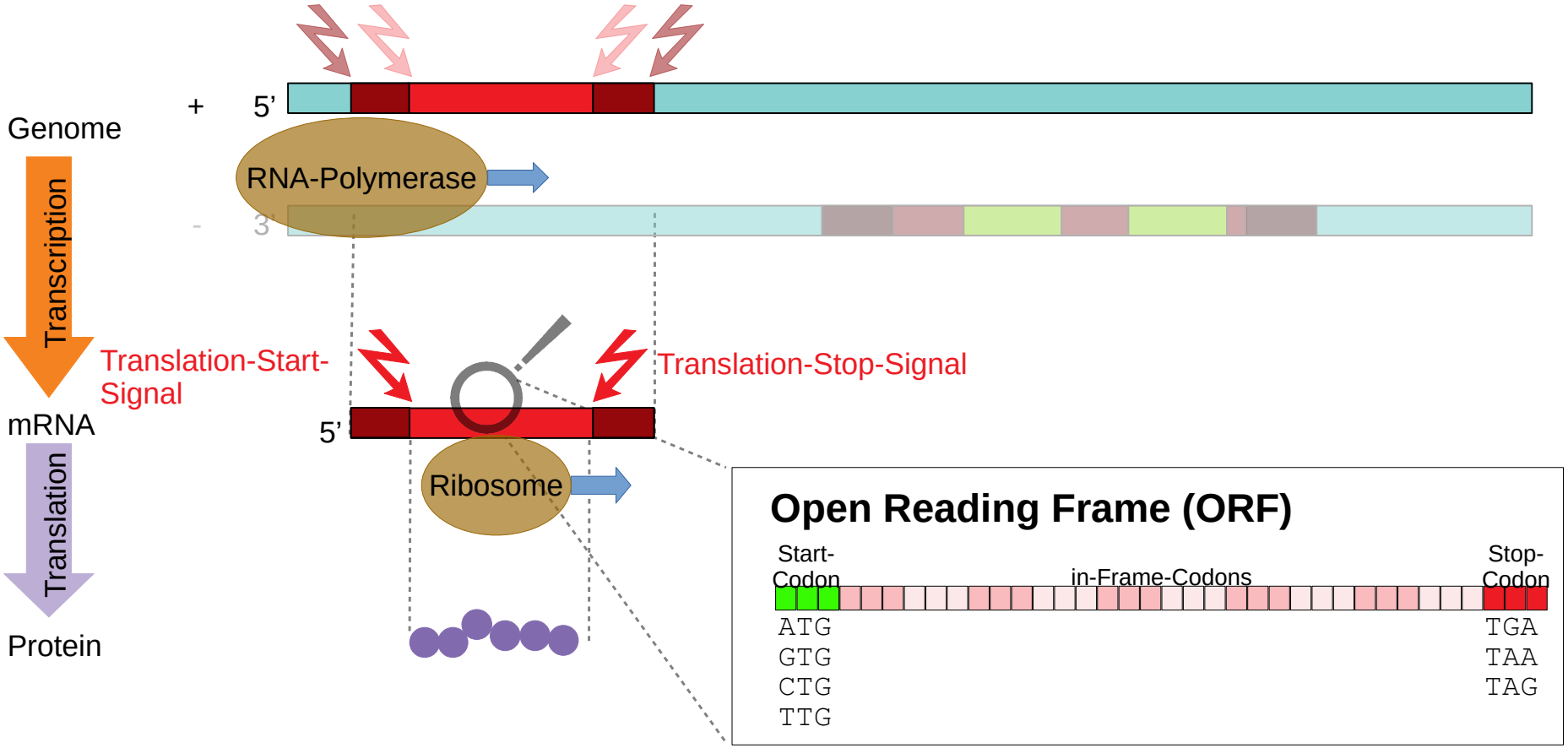
How does a cell recognize protein-coding genes?

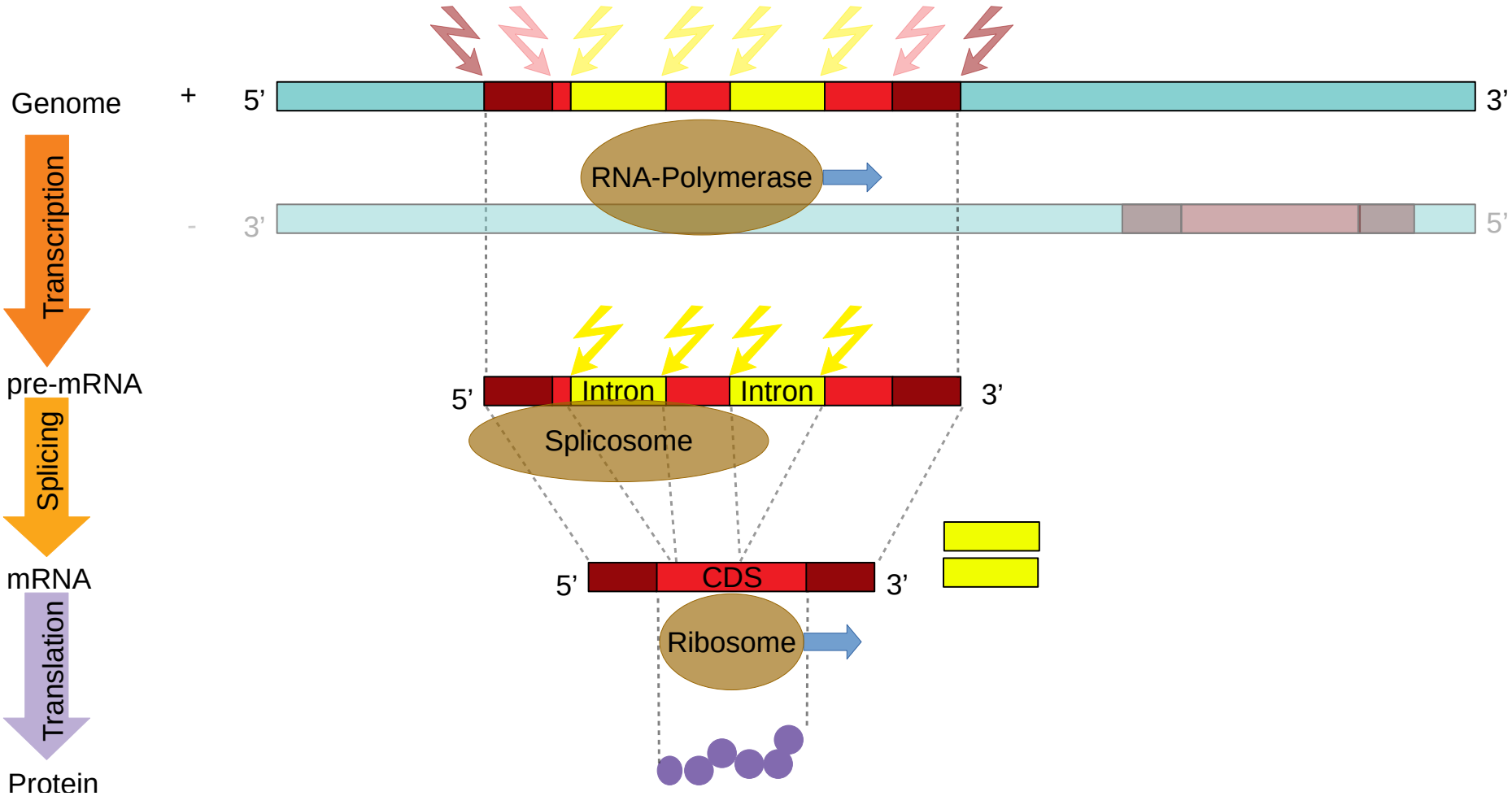
Prokaryotes & Eukaryotes*



- 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

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The Genome Annotation Problem

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

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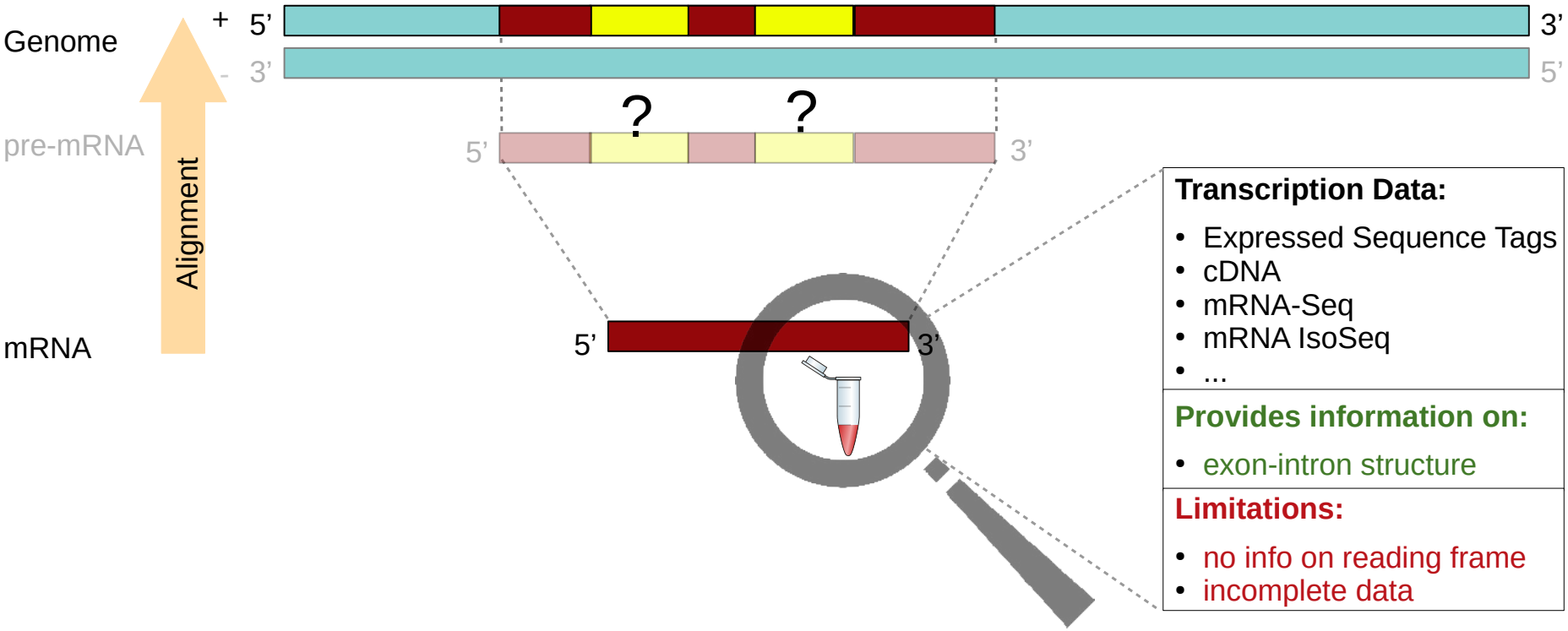
What aids in the identification of genes in genomes?

Evidence data from transcription



Transcription Data:

- Expressed Sequence Tags
- cDNA
- mRNA-Seq
- mRNA IsoSeq
- ...

Provides information on:

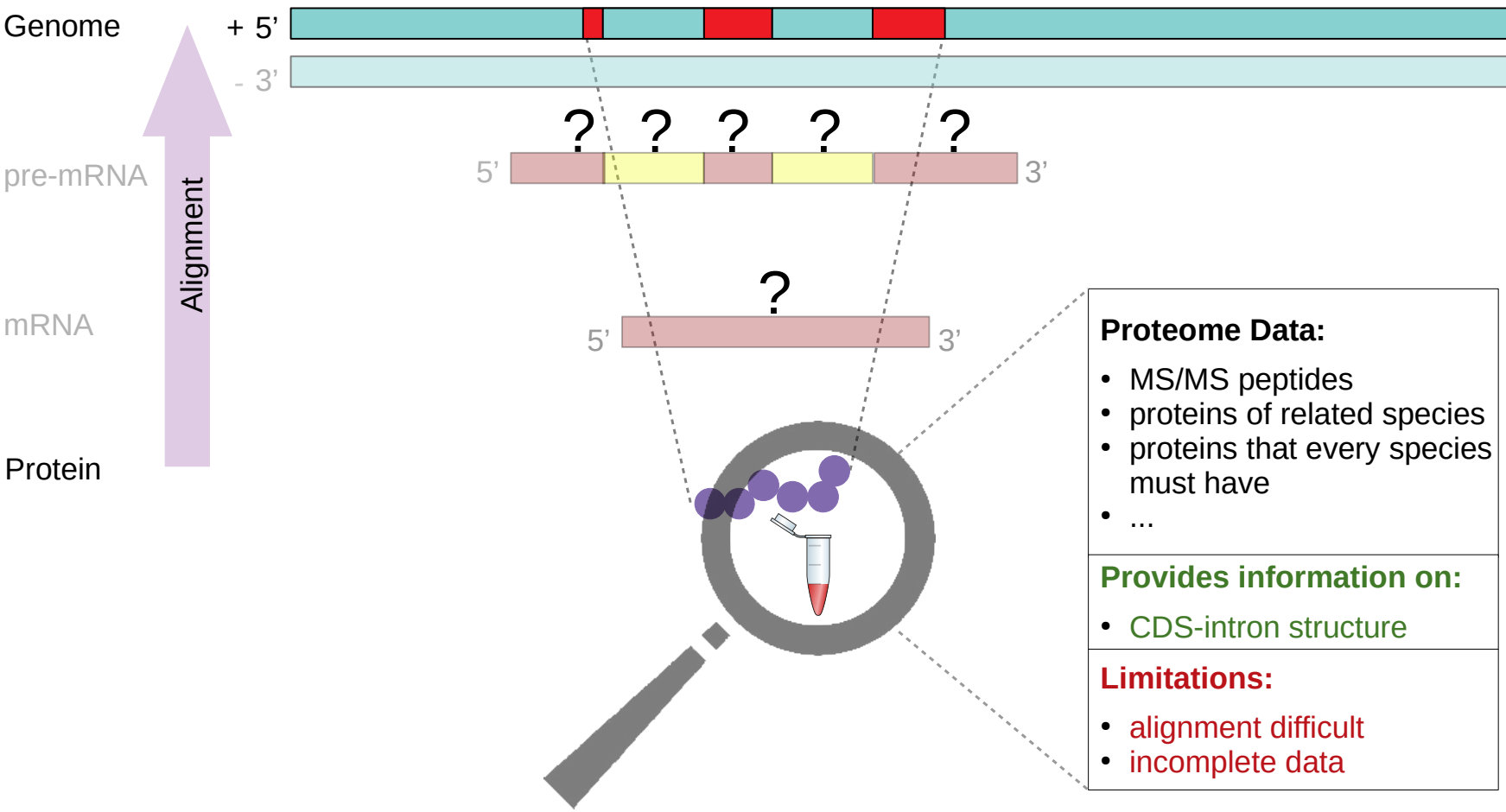
- exon-intron structure

Limitations:

- no info on reading frame
- incomplete data

What aids in the identification of genes in genomes?

Evidence data from translation



Proteome Data:

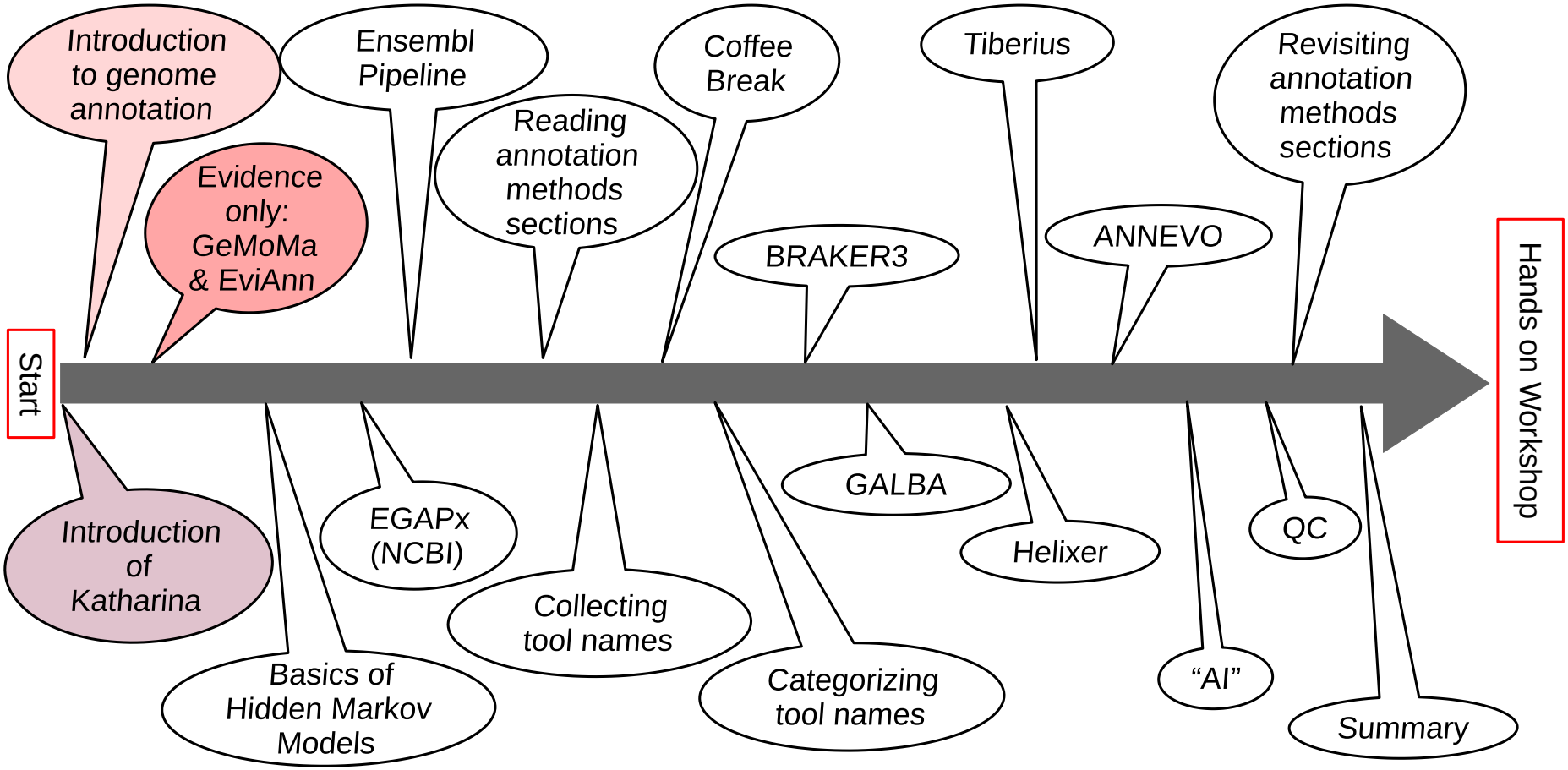
- MS/MS peptides
- proteins of related species
- proteins that every species must have
- ...

Provides information on:

- CDS-intron structure

Limitations:

- alignment difficult
- incomplete data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Genome Annotation Tools that Rely on Evidence Data

GeMoMa

- Java tool
- required inputs:
  - genome file
  - genome files of close relative species
  - annotation files from (several) close relative species
  - optional: transcriptome data

Keilwagen et al. (2016); Keilwagen et al. (2018)

EviAnn Input Data

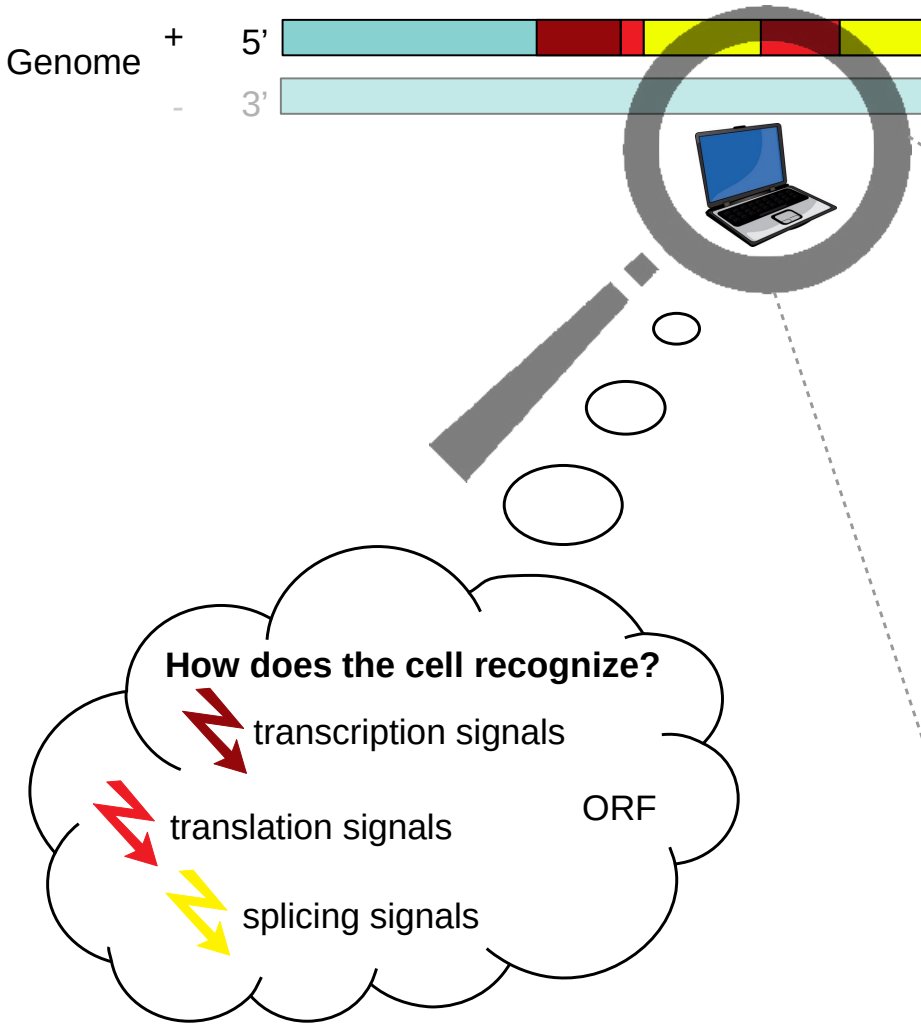
- Perl pipeline
- required inputs:
  - genome file
  - protein sequences from (several) close relative species
  - transcriptome data

Zimin *et al.* (2025)

\+ high accuracy if annotations of very close relatives are available
\- cannot predict genes that have no evidence

What aids in the identification of genes in genomes?

Mathematical models



Mathematical models:

- **Hidden Markov Models**
  (e.g. GeneMark, AUGUSTUS)
- dynamic programming
- Support Vector Machines
- neural networks
- decision tree systems
- ...

Provide information on:

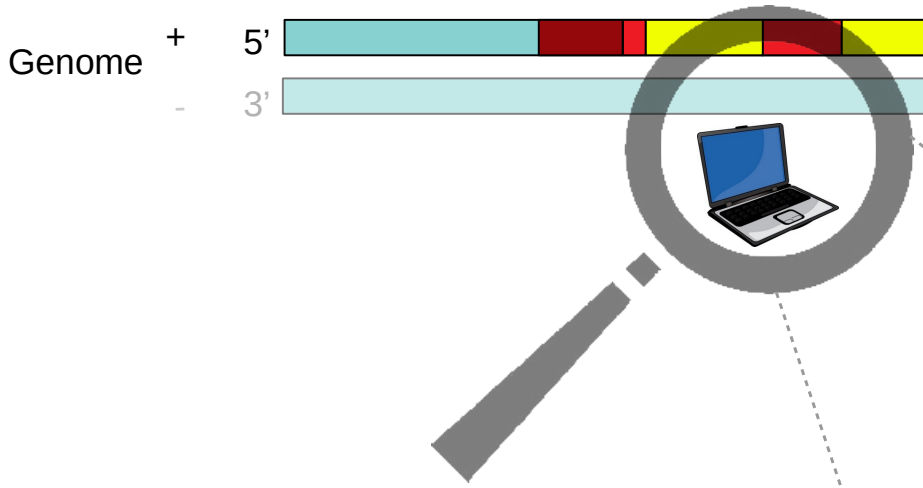
- complete gene structures (sometimes incl. UTRs)

Limitations

- *predictions* may be wrong
- models use **parameters** that have to be trained

What aids in the identification of genes in genomes?

Mathematical models



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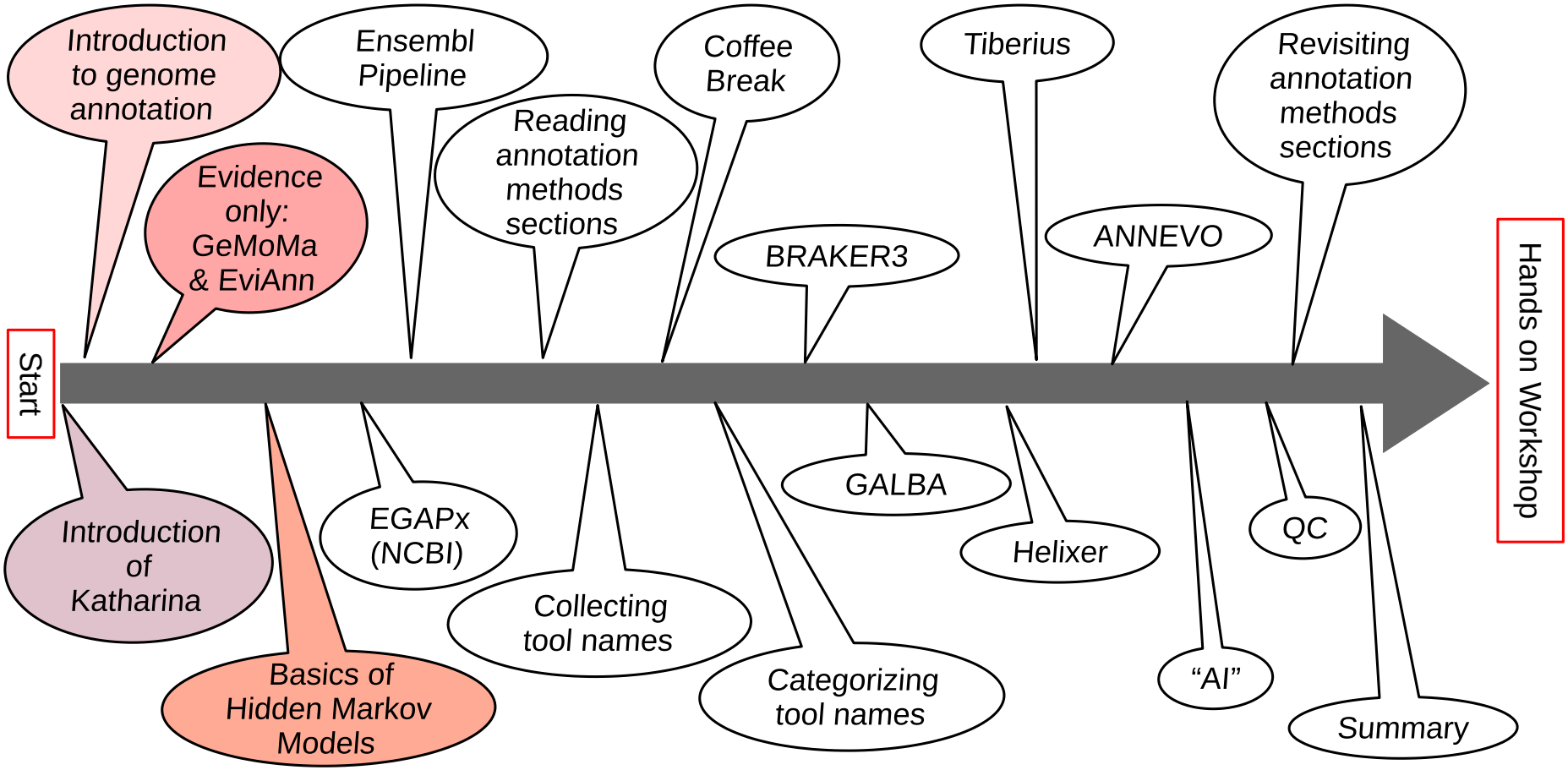
A **Hidden Markov Model** can read the genome sequence from left to right and, through knowledge of signals for transcription and translation, assign a probable state to each nucleotide (e.g., intergenic region or CDS).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Simplifications

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

Input: “Genome sequence”

e.g. AABBBAB

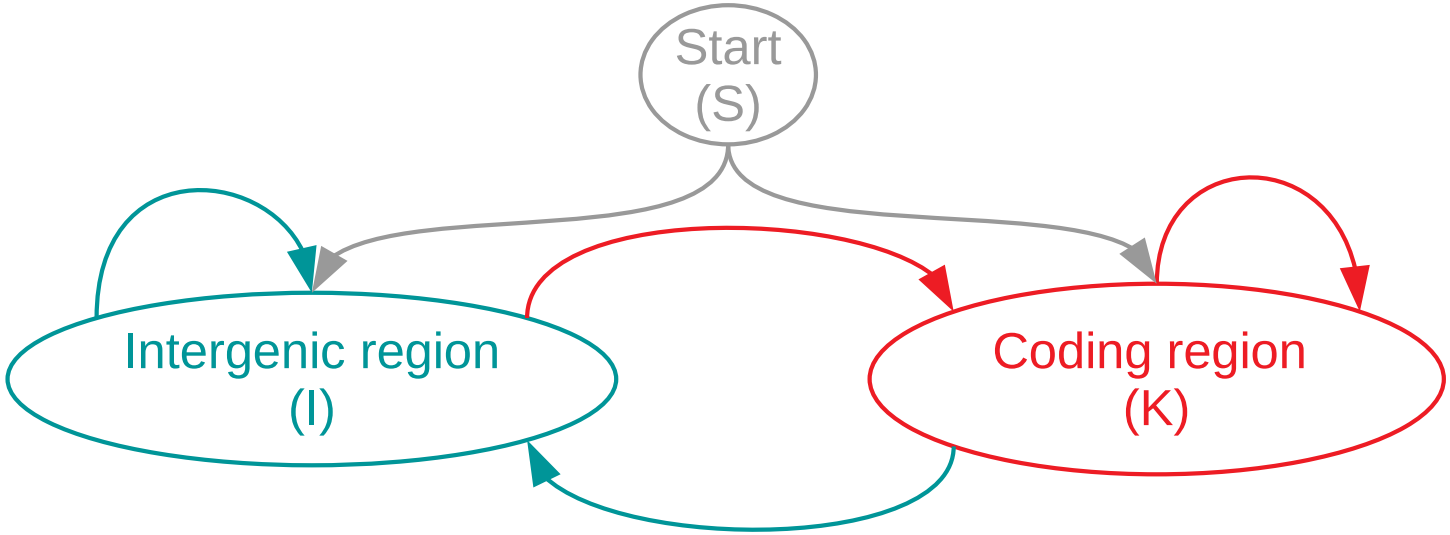
Goal: “Most likely path through hidden states”

```
e.g. AABBBAA
or   IIKKIKI     P(path) = 0.3%
```

Basis of highly accurate gene prediction tools

Hidden Markov Model

Hidden states



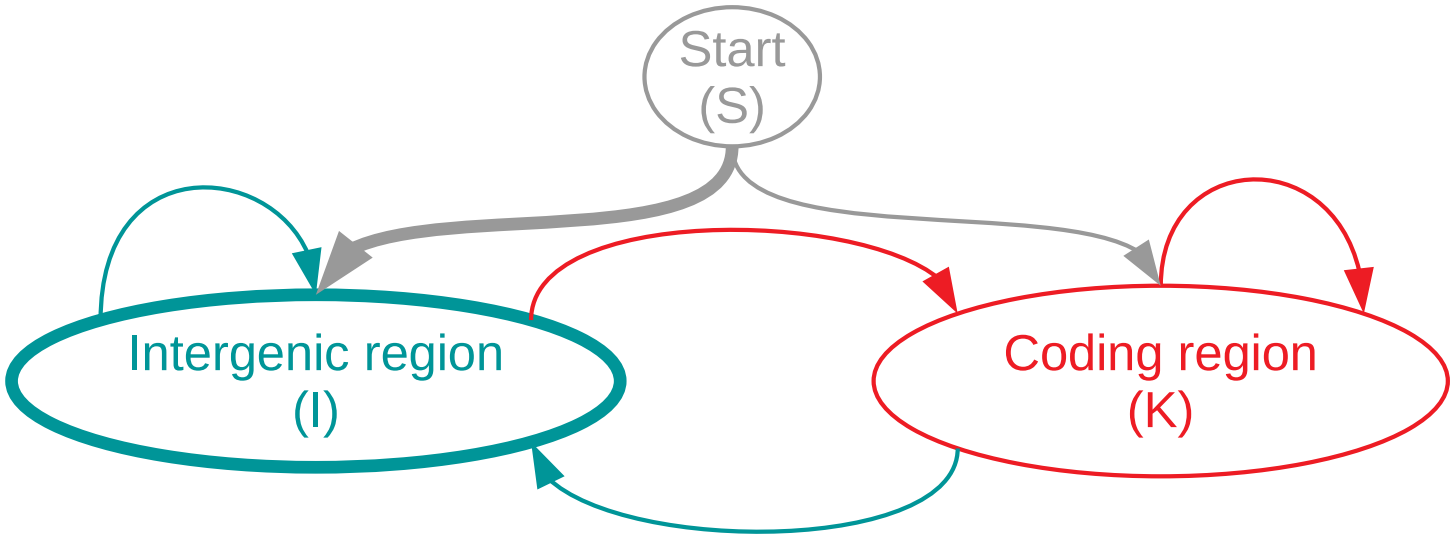
A possible 'state path' for the genome sequence:

AABBBAA

Basis of highly accurate gene prediction tools

Hidden Markov Model

Hidden states



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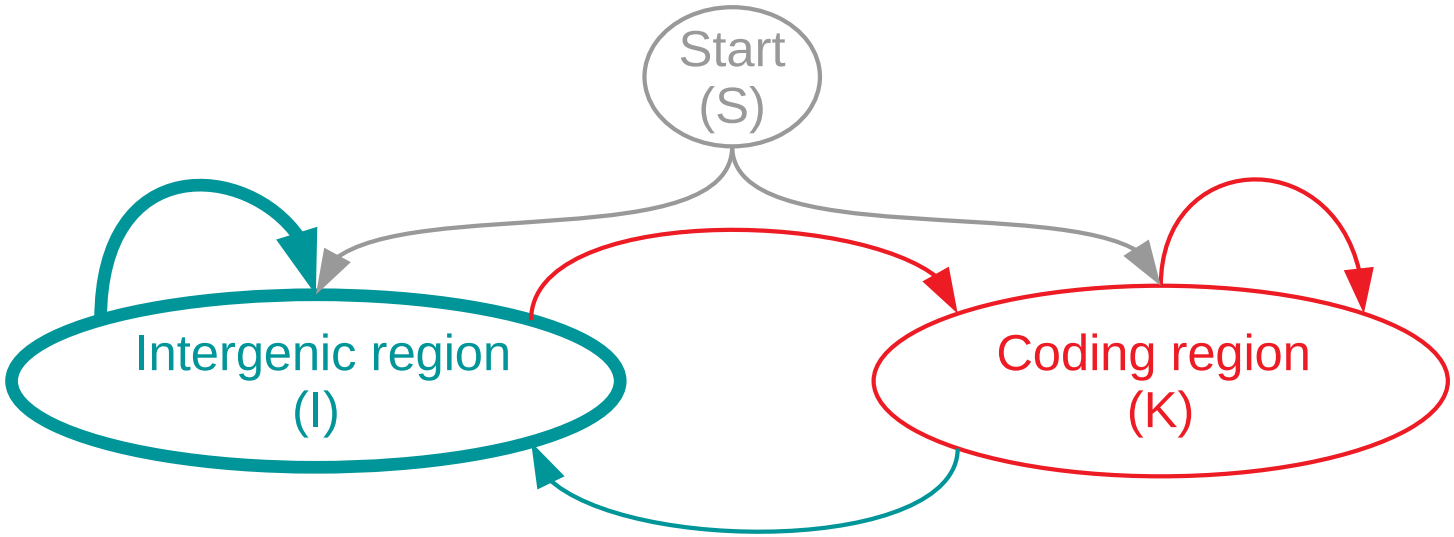

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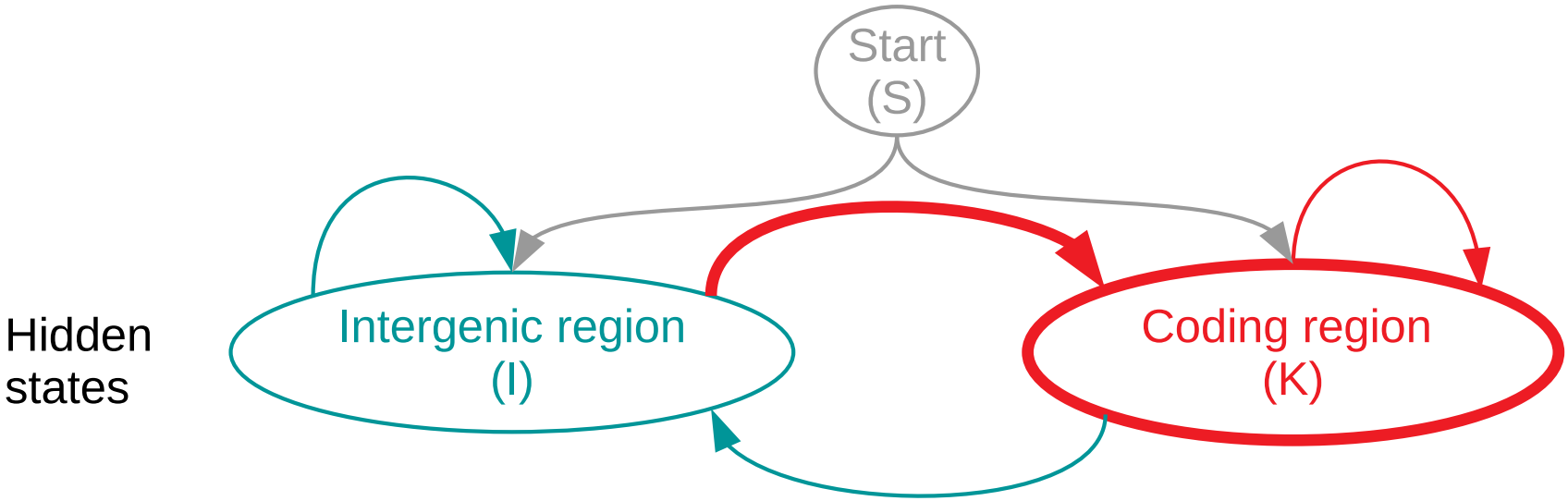
II

Basis of highly accurate gene prediction tools

Hidden Markov Model



A possible 'state path' for the genome sequence:

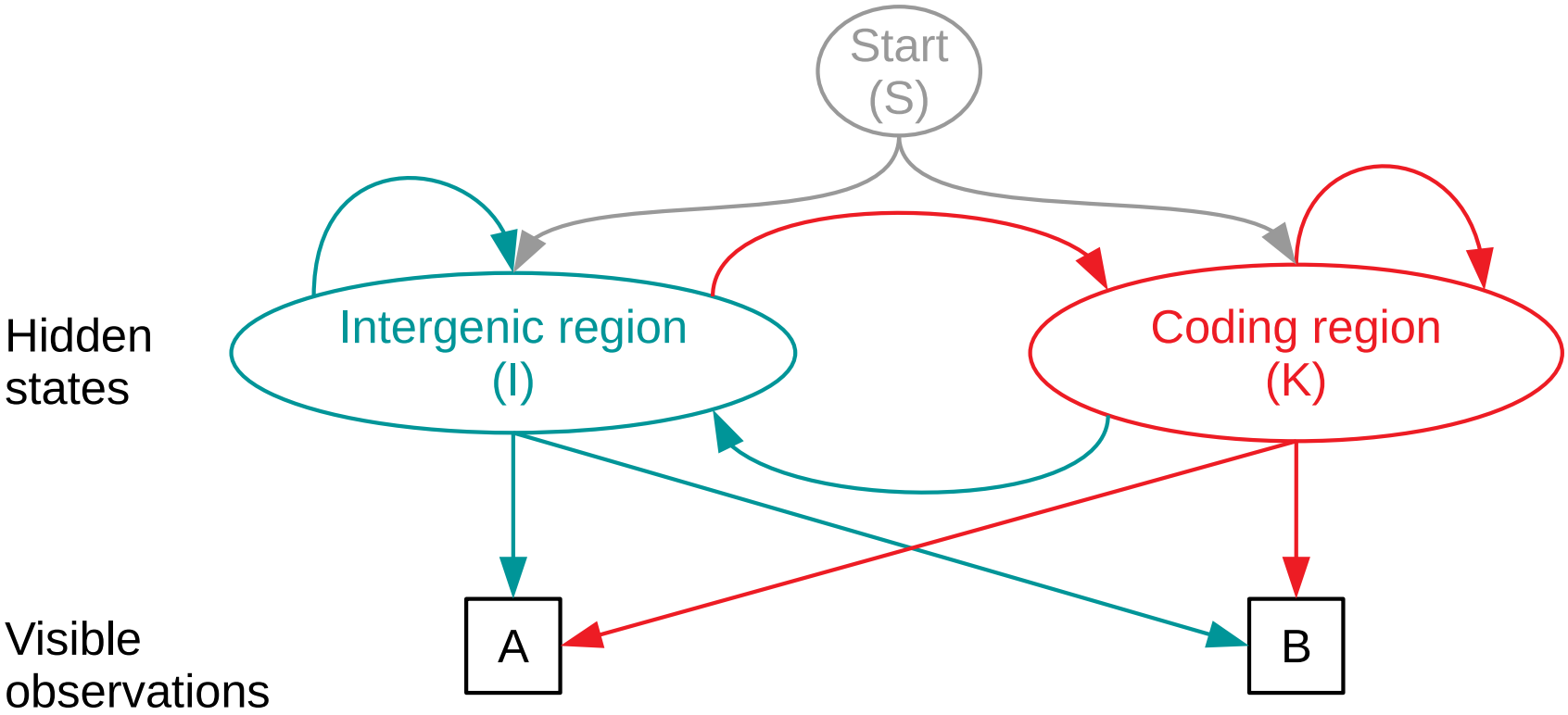
```
AABBBAA
IIK...
```

Model properties

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

Basis of highly accurate gene prediction tools

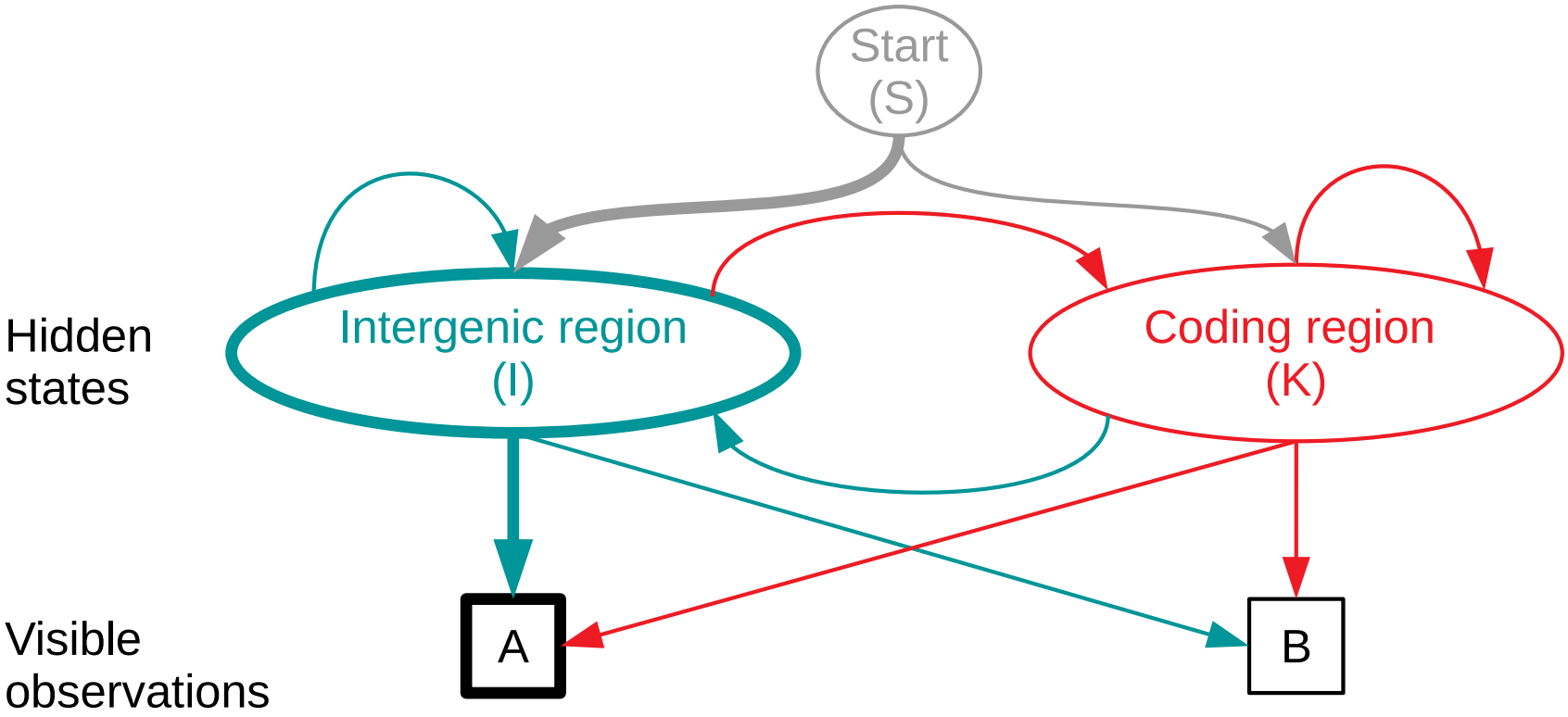
Hidden Markov Model



A possible ‘state path’ for the genome sequence:

Basis of highly accurate gene prediction tools

Hidden Markov Model

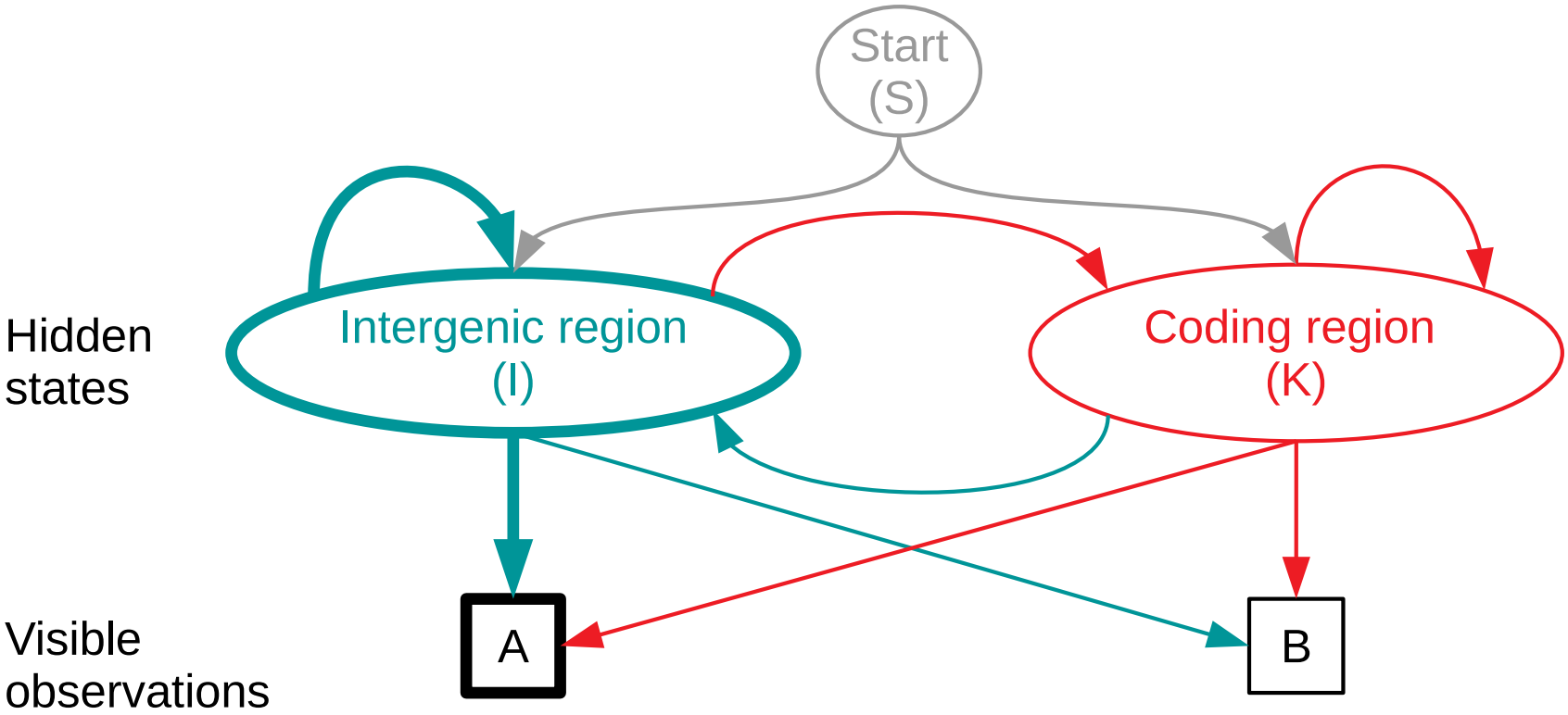


A possible ‘state path’ for the genome sequence:

A
I

Basis of highly accurate gene prediction tools

Hidden Markov Model

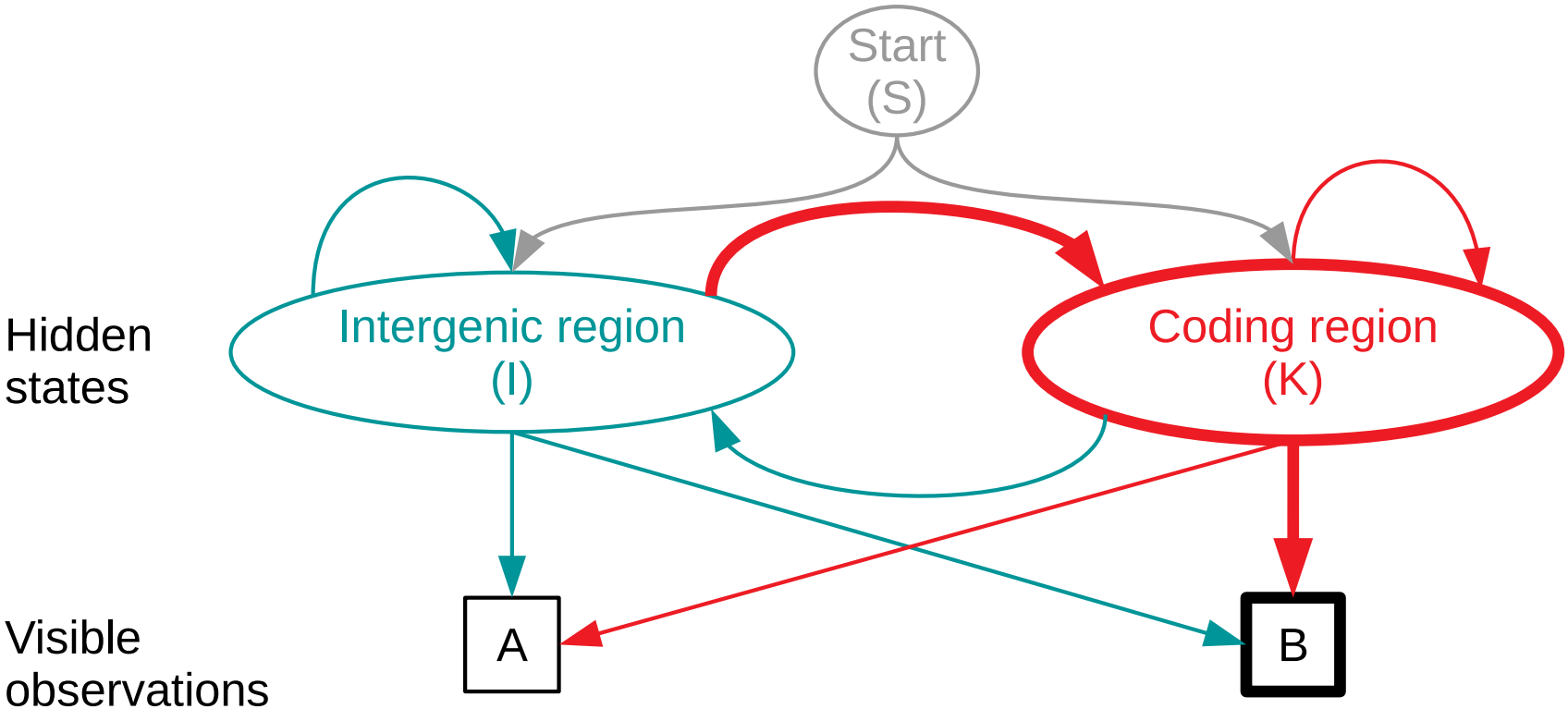


A possible 'state path' for the genome sequence:

AA
II

Basis of highly accurate gene prediction tools

Hidden Markov Model

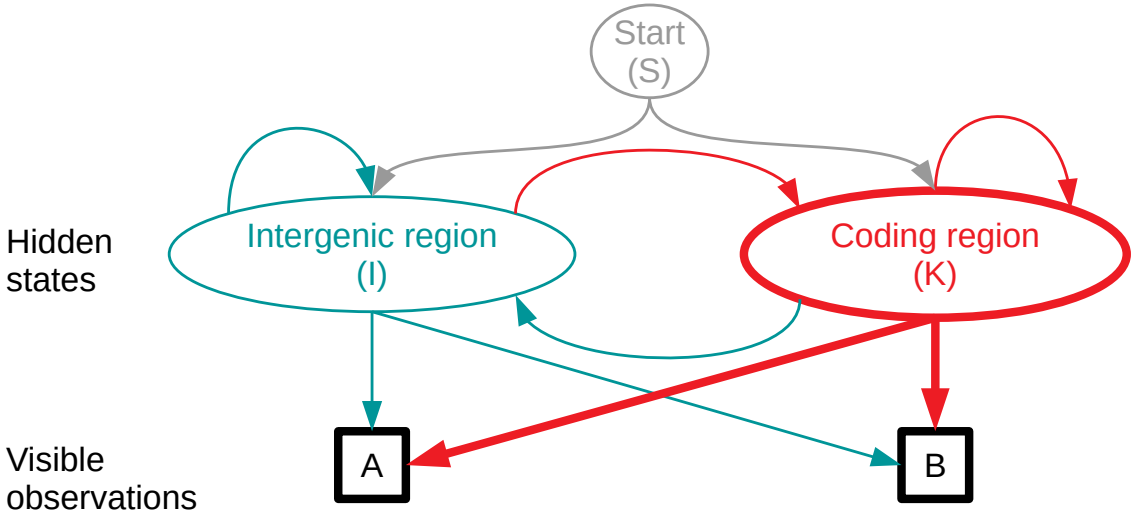


A possible 'state path' for the genome sequence:

```
AAB...
IIK...
```

Basis of highly accurate gene prediction tools

Hidden Markov Model

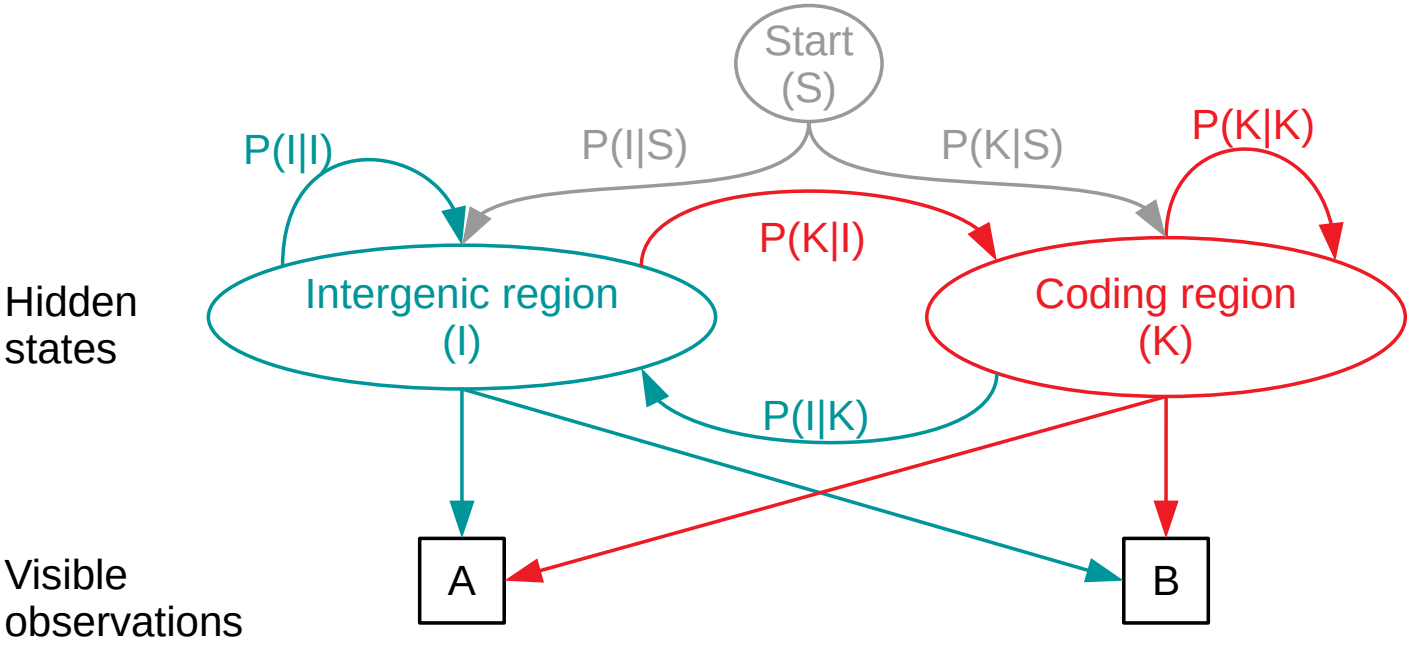


Model properties

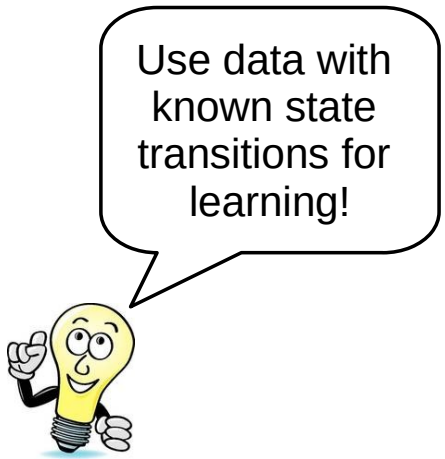
1. The current value of the hidden state depends exclusively on the state of its predecessor.
2. The current value of the visible observation depends exclusively on the value of the current, hidden state.

Basis of highly accurate gene prediction tools

Hidden Markov Model

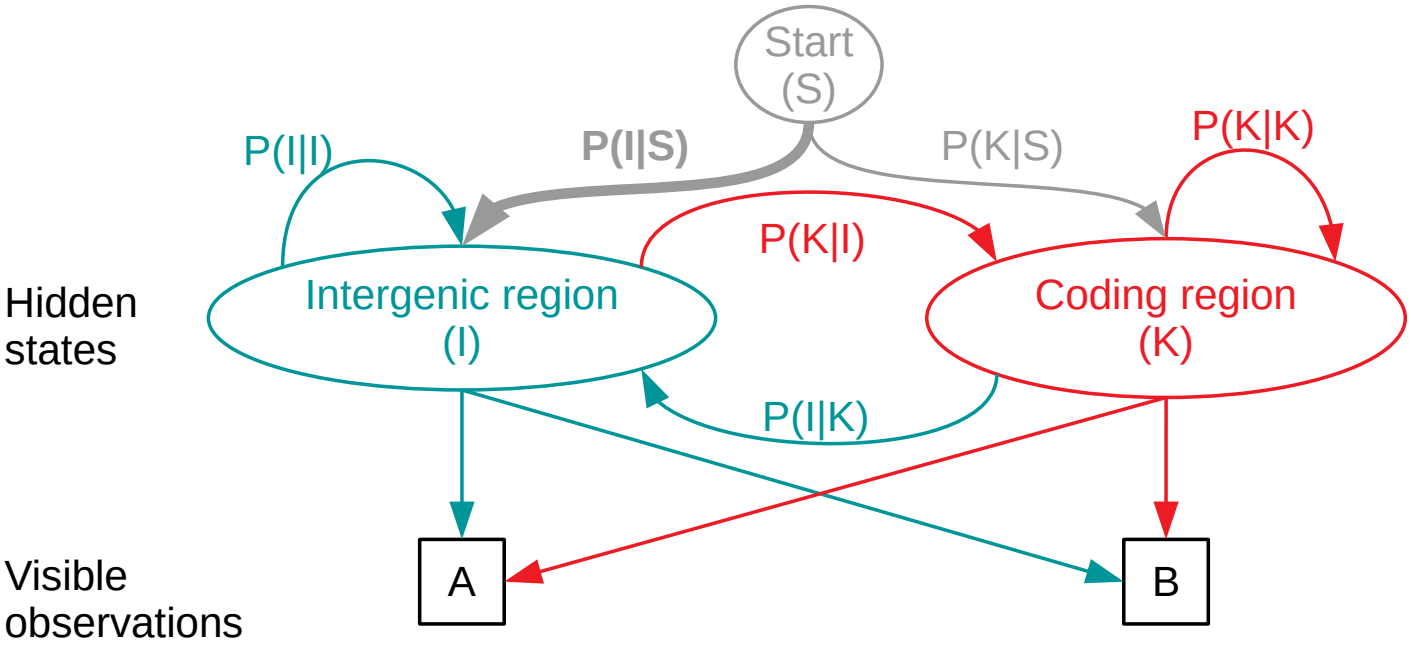


How likely are the state transitions?



Basis of highly accurate gene prediction tools

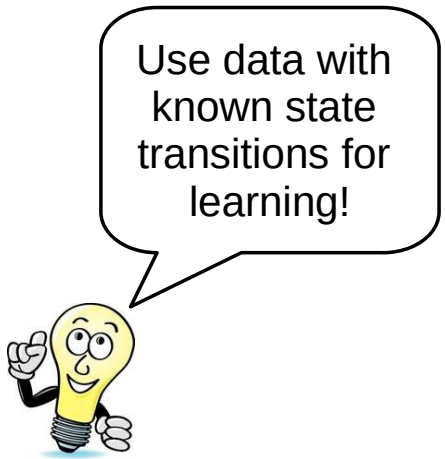
Hidden Markov Model



Training data:

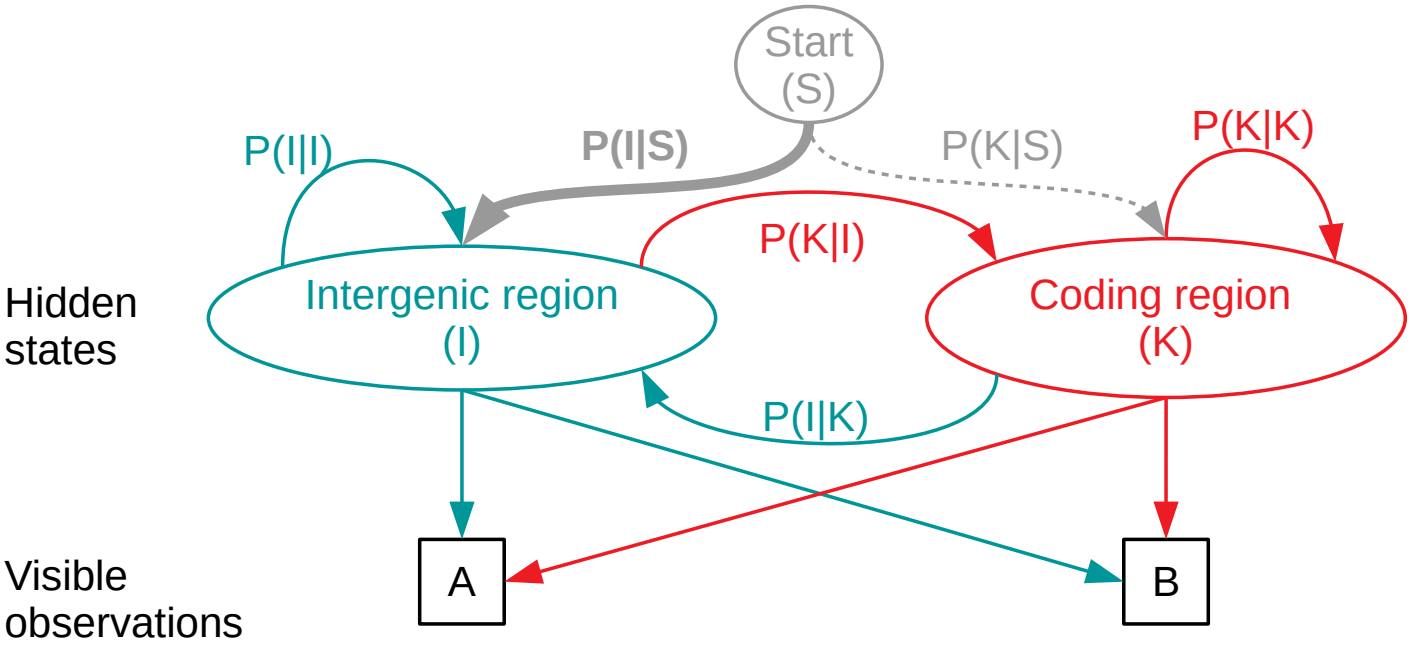
AABABA
IKKIII

Start probability
P(I|S) = ?



Basis of highly accurate gene prediction tools

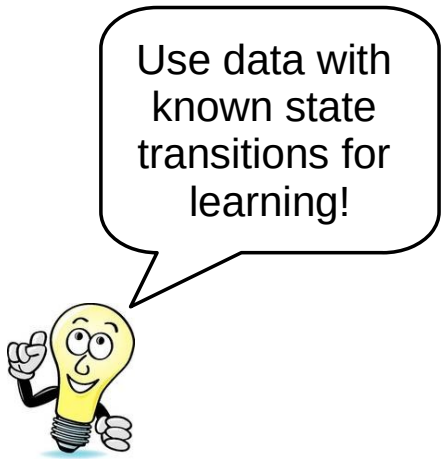
Hidden Markov Model



Training data:

AABABA
IKKIII
+

Start probability
P(I|S) = 1



Basis of highly accurate gene prediction tools

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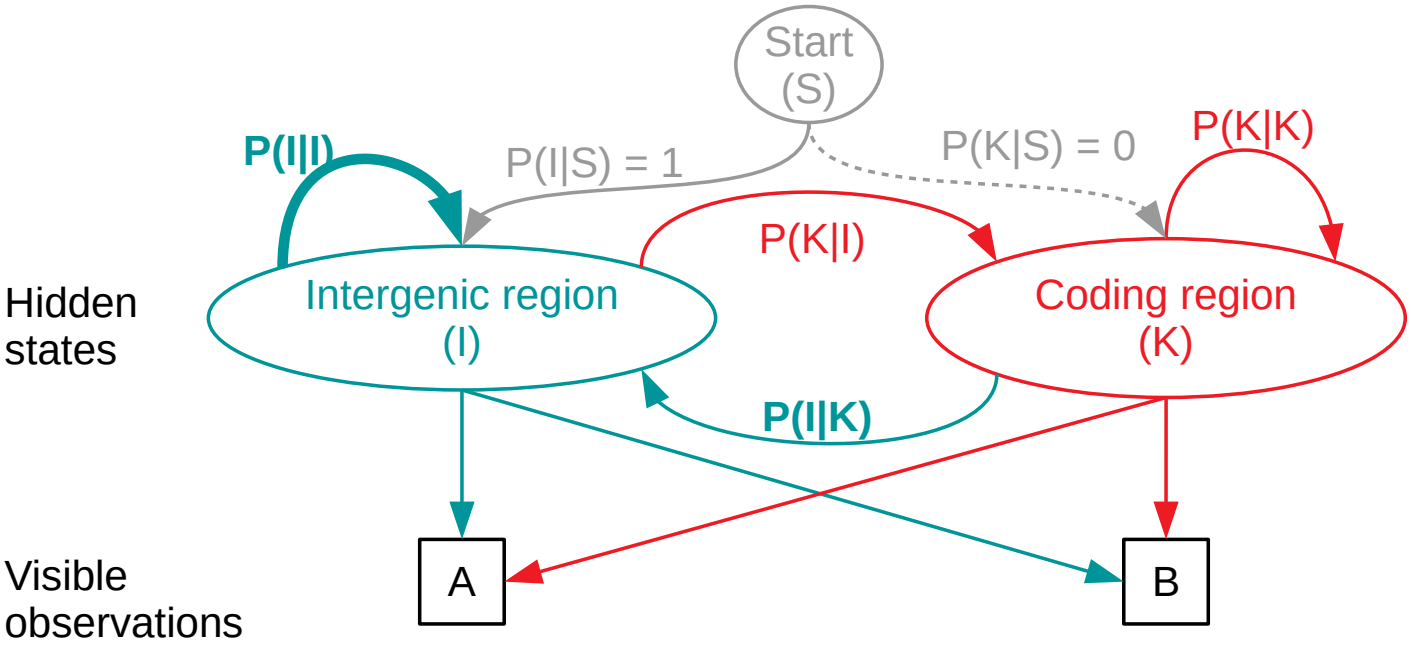
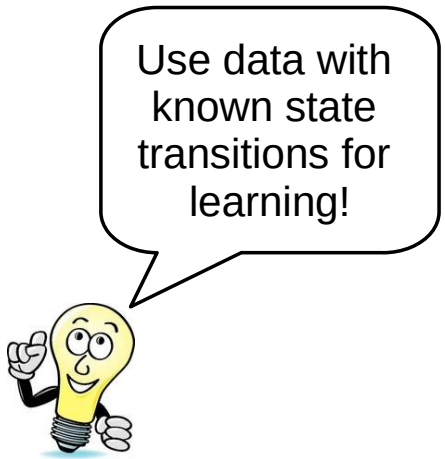


Training data:

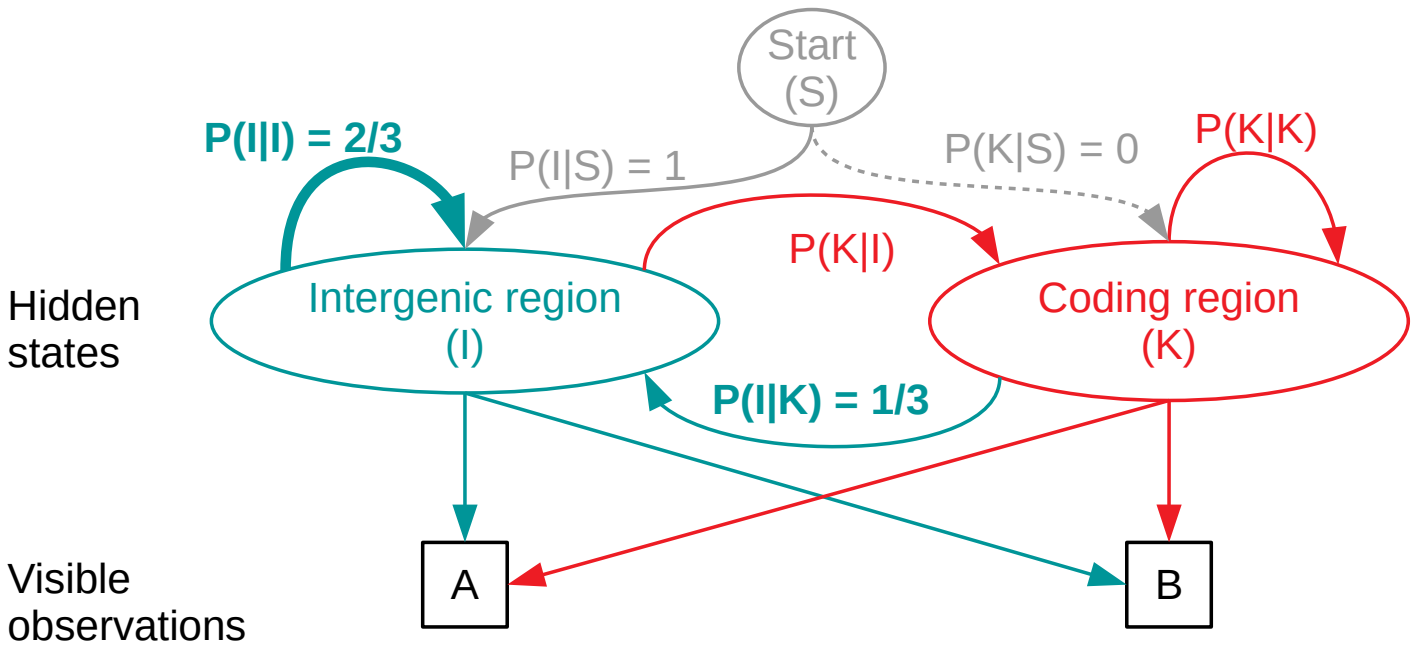
AABABA
IKKIII

P(I|I) = ?



Basis of highly accurate gene prediction tools

Hidden Markov Model



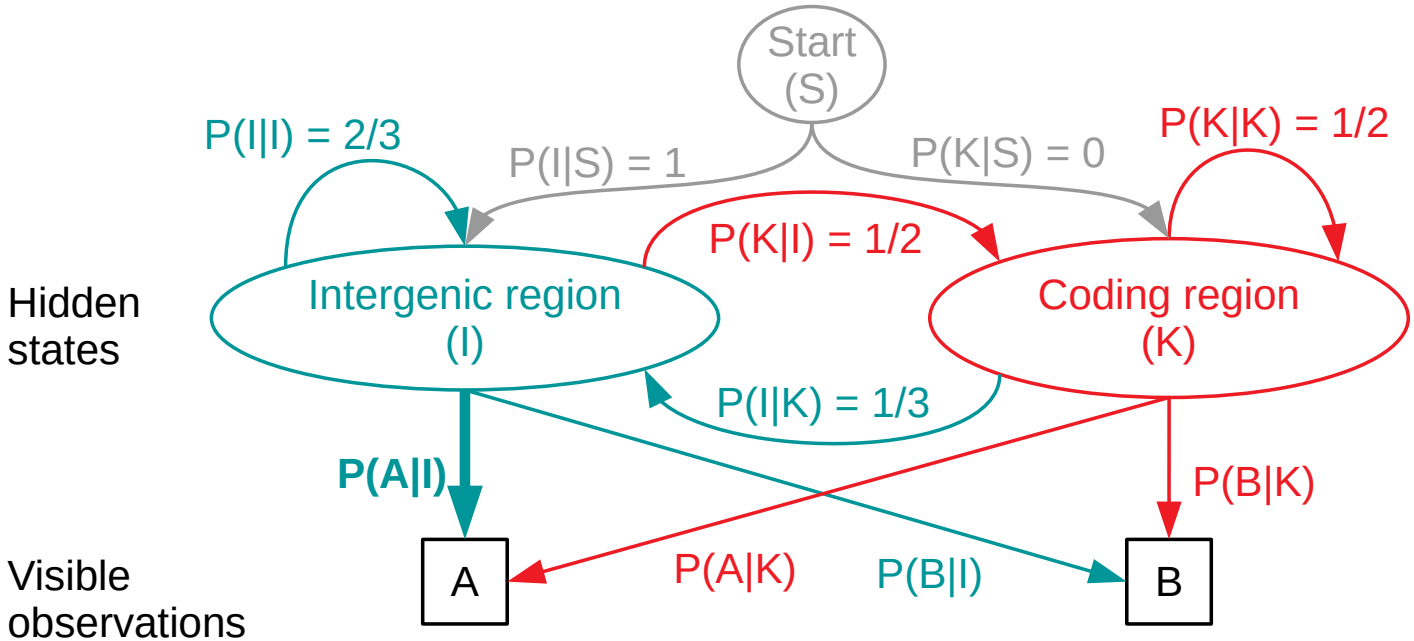
Training data:

AABABA
IKKIII
-++

P(I|I) = 2/3 P(I|K) = 1 – P(I|I) = 1/3

Basis of highly accurate gene prediction tools

Hidden Markov Model



How likely are the observations?

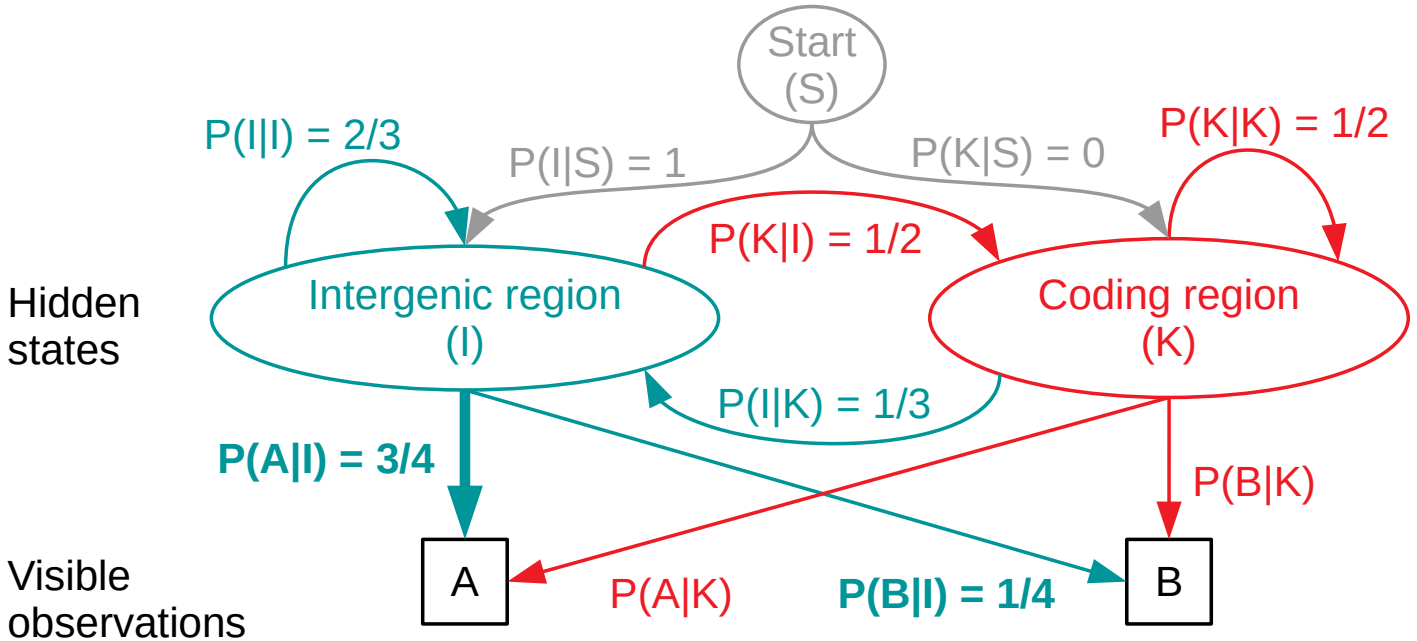
AABABA
IKKIII

P(A|I) = ?

Use data with known "emissions" for learning!

Basis of highly accurate gene prediction tools

Hidden Markov Model



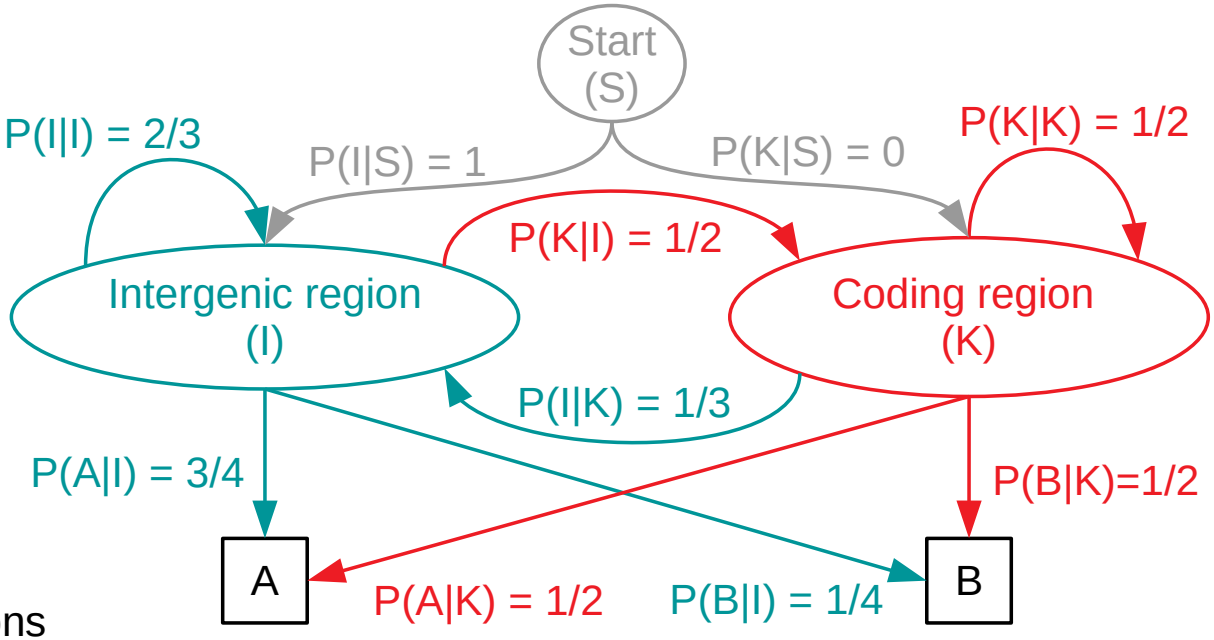
How likely are the observations?

AABABA
IKKIII
+ +-+

P(A|I) = ¾ P(B|I) = 1 – P(A|I) = 1 – ¾ = ¼

Basis of highly accurate gene prediction tools

Hidden Markov Model

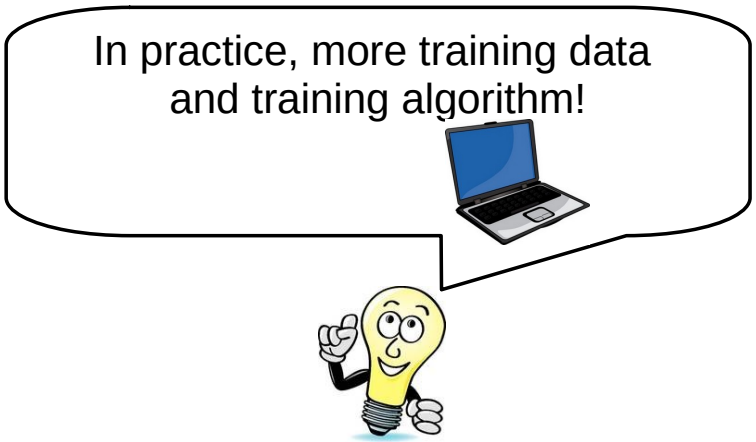


Hidden states

Visible observations (emissions)

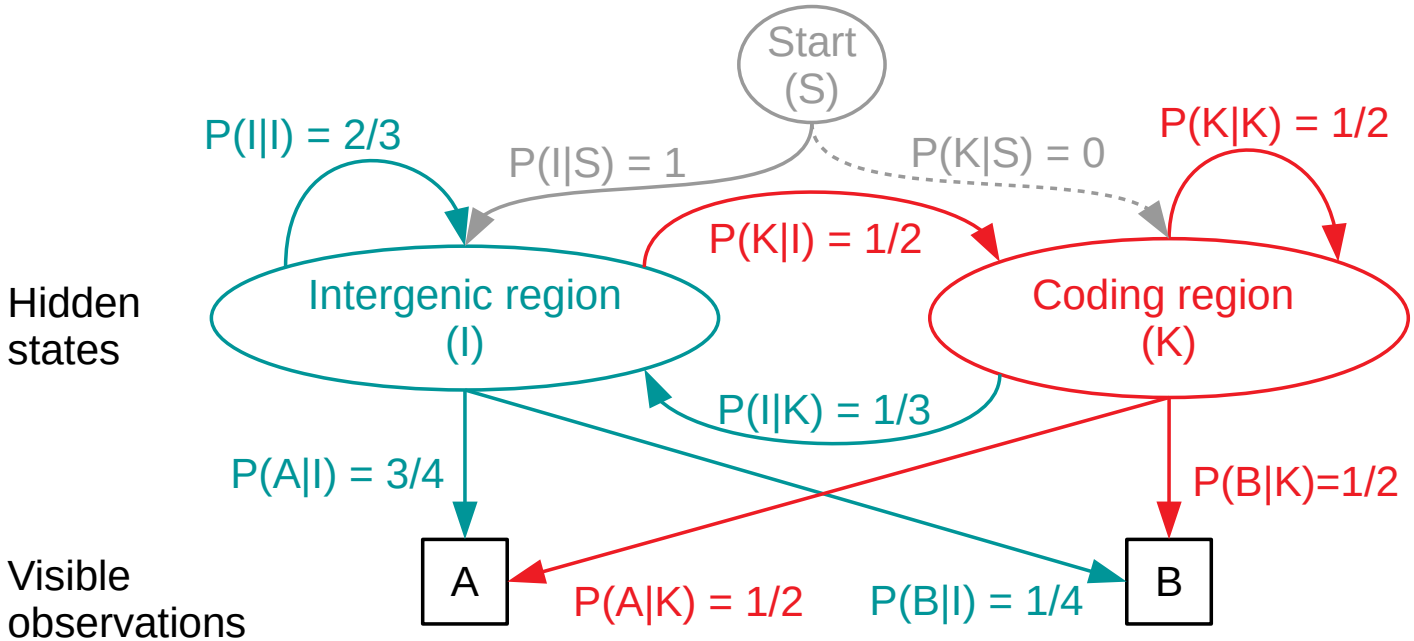
Training data:

AABABA
IKKIII



Basis of highly accurate gene prediction tools

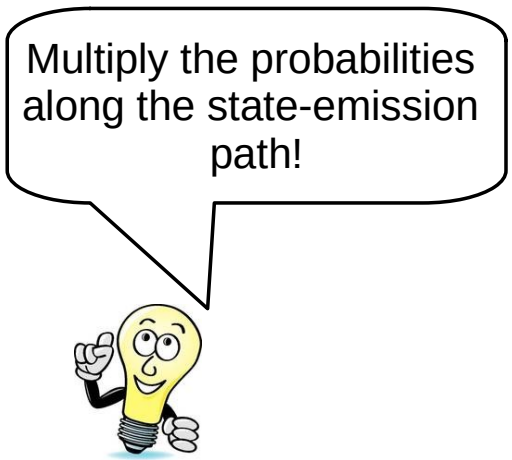
Hidden Markov Model



How likely is a given state-emission path?

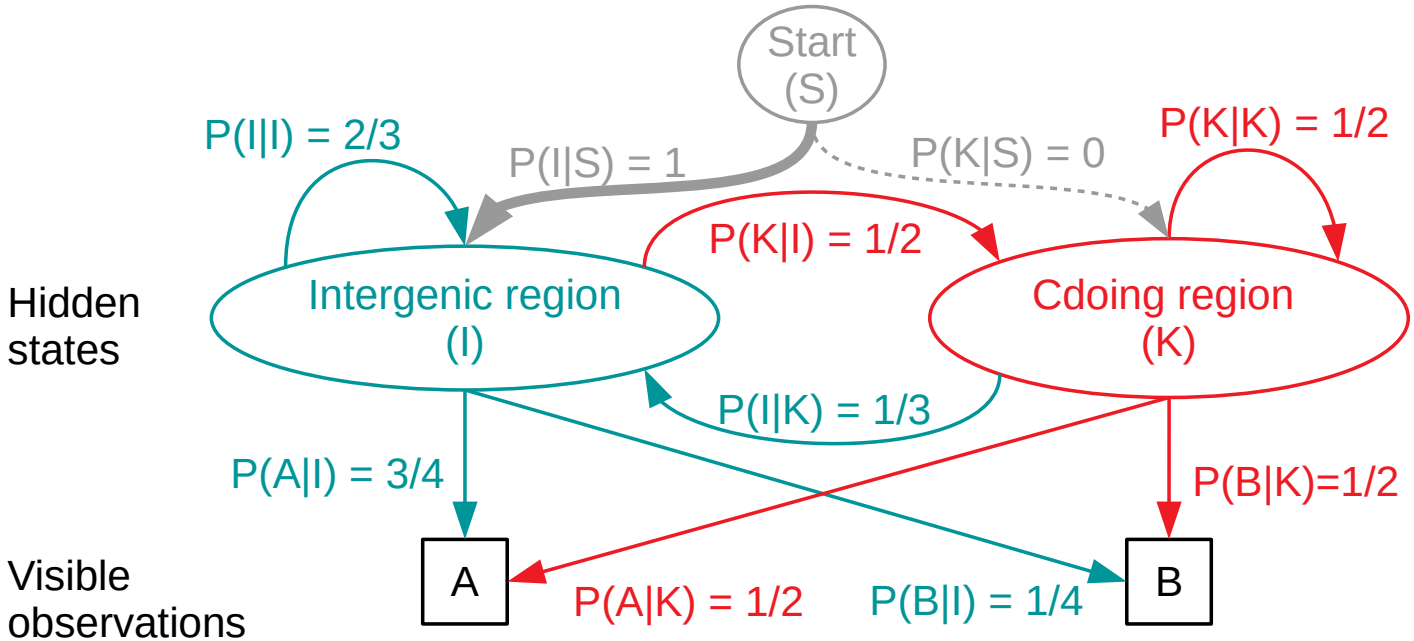
```
Path = AAB
       IKK

P(Path) = ?
```



Basis of highly accurate gene prediction tools

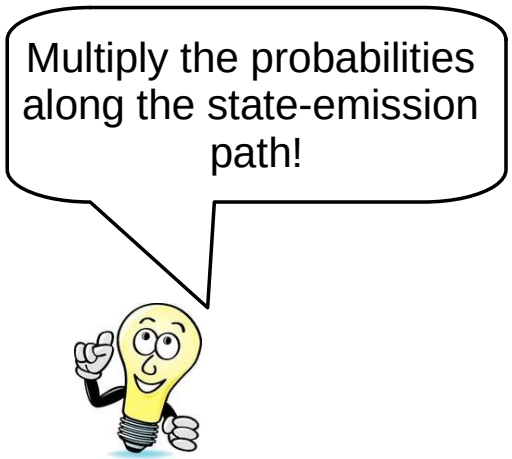
Hidden Markov Model



How likely is a given state-emission path?

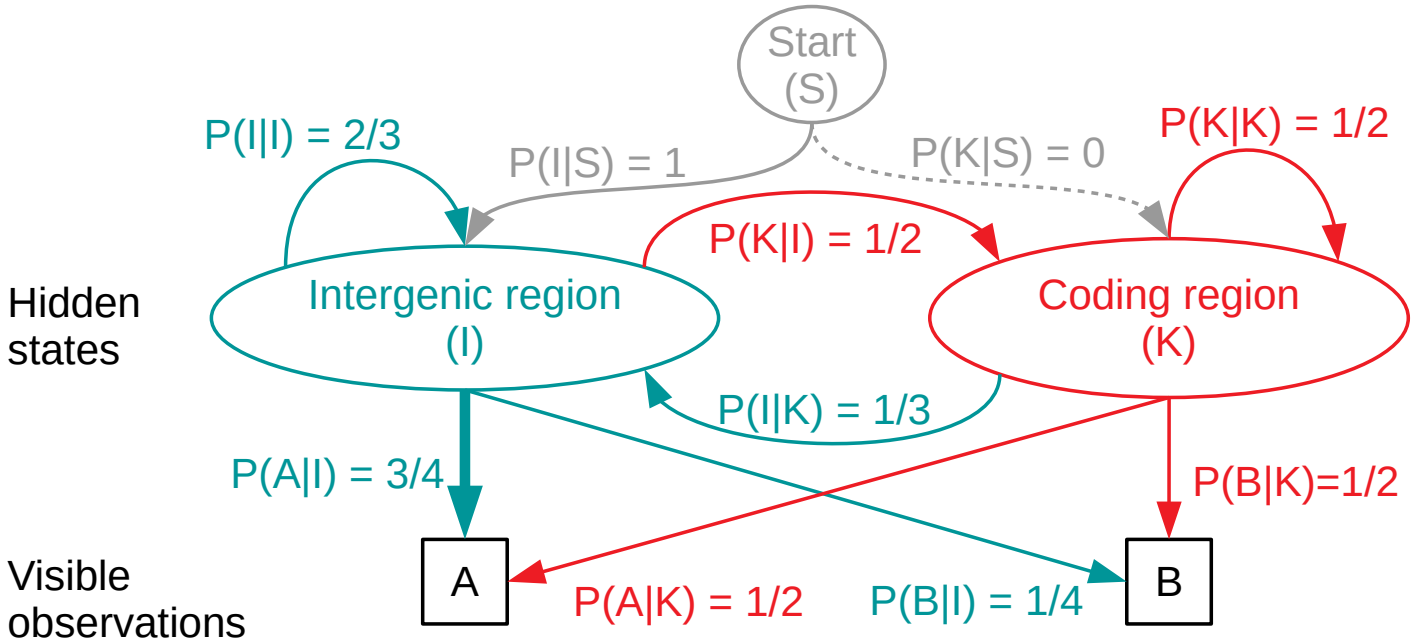
```
Path = AAB
       IKK

P(Path) = P(I|S)
```



Basis of highly accurate gene prediction tools

Hidden Markov Model



How likely is a given state-emission path?

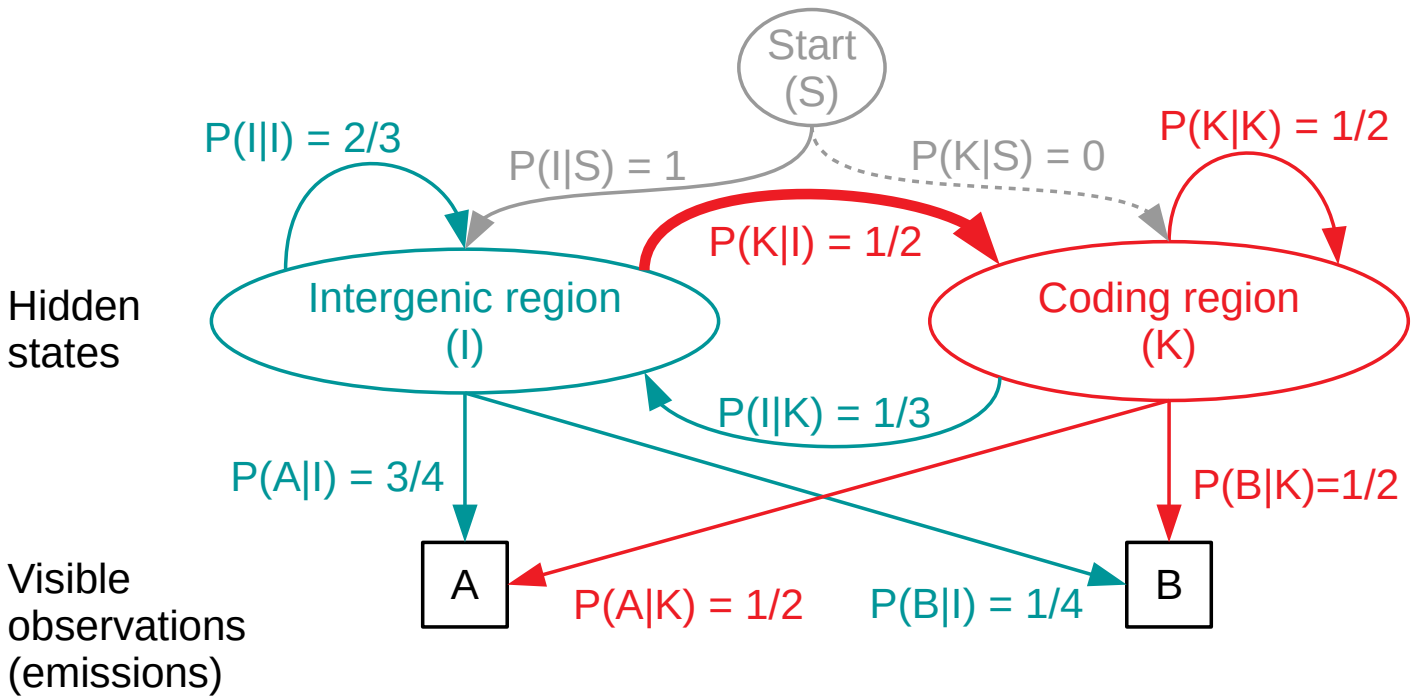
```
Path = AAB
       IKK

P(Path) = P(I|S)*P(A|I)
```

Multiply the probabilities along the state-emission path!

Basis of highly accurate gene prediction tools

Hidden Markov Model



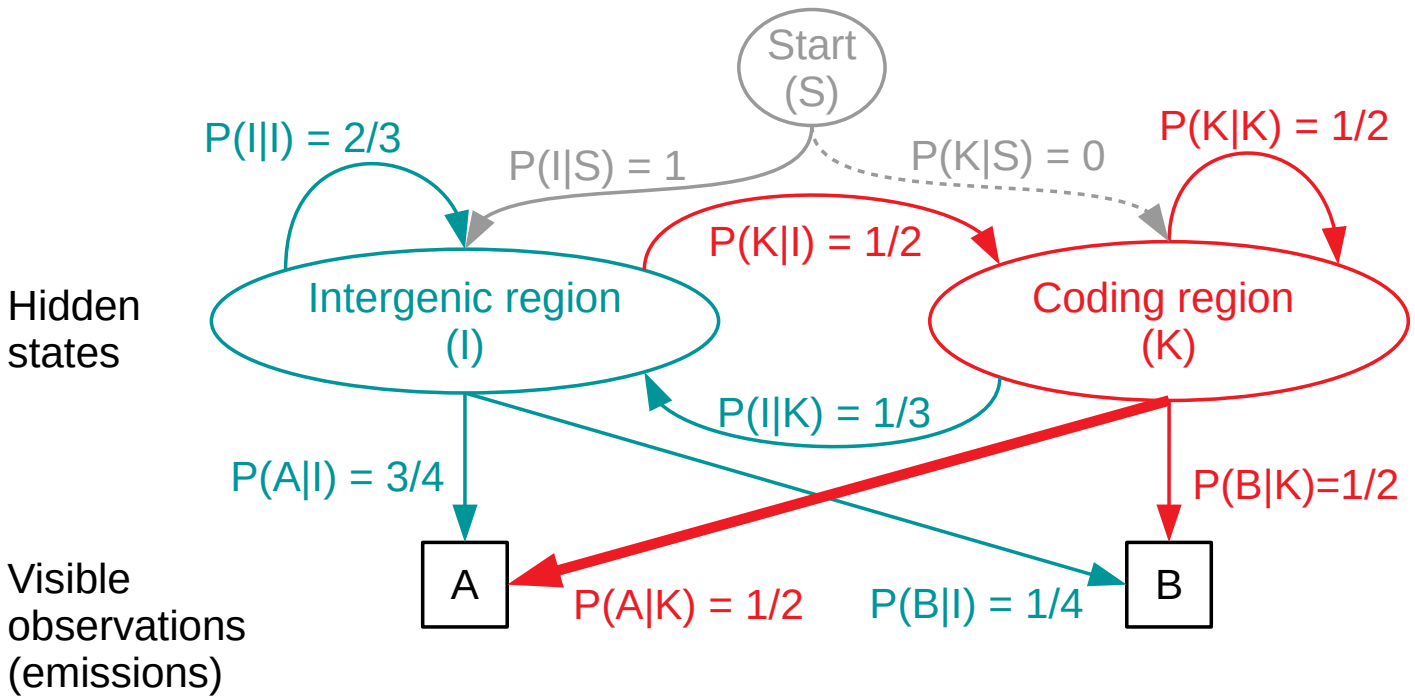
How likely is a given state-emission path?

```
Path = AAB
       IKK

P(Path) = P(I|S)*P(A|I)*P(K|I)
```

Basis of highly accurate gene prediction tools

Hidden Markov Model



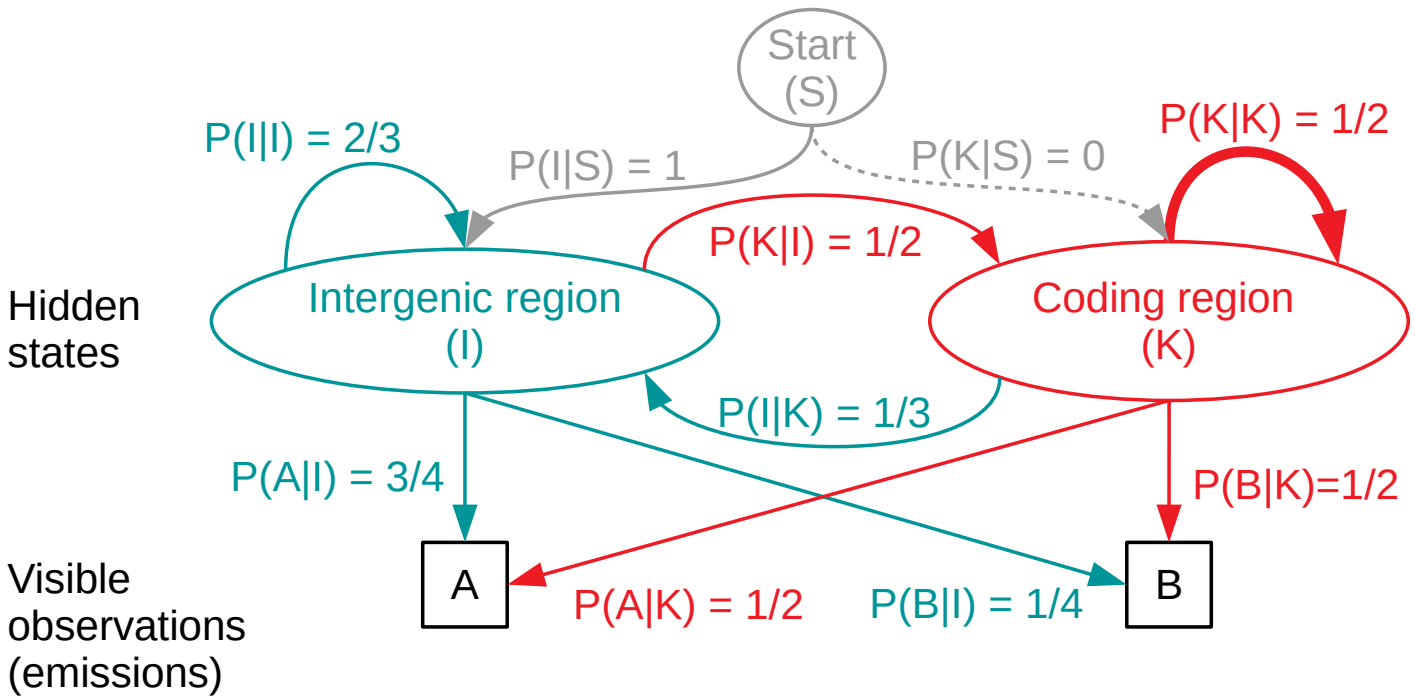
How likely is a given state-emission path?

```
Path = AAB
       IKK

P(Path) = P(I|S)*P(A|I)*P(K|I)*P(A|K)
```

Basis of highly accurate gene prediction tools

Hidden Markov Model



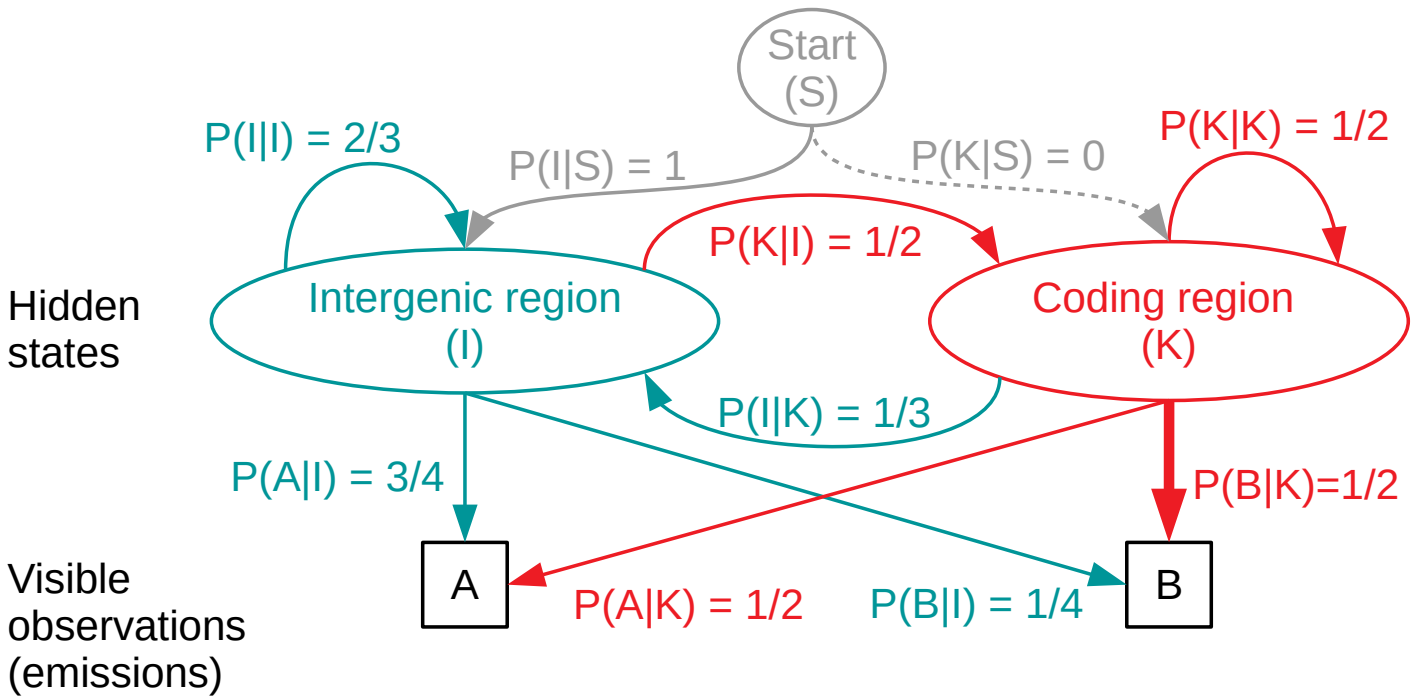
How likely is a given state-emission path?

```
Path = AAB
       IKK

P(Path) = P(I|S)*P(A|I)*P(K|I)*P(A|K)*P(K|K)
```

Basis of highly accurate gene prediction tools

Hidden Markov Model



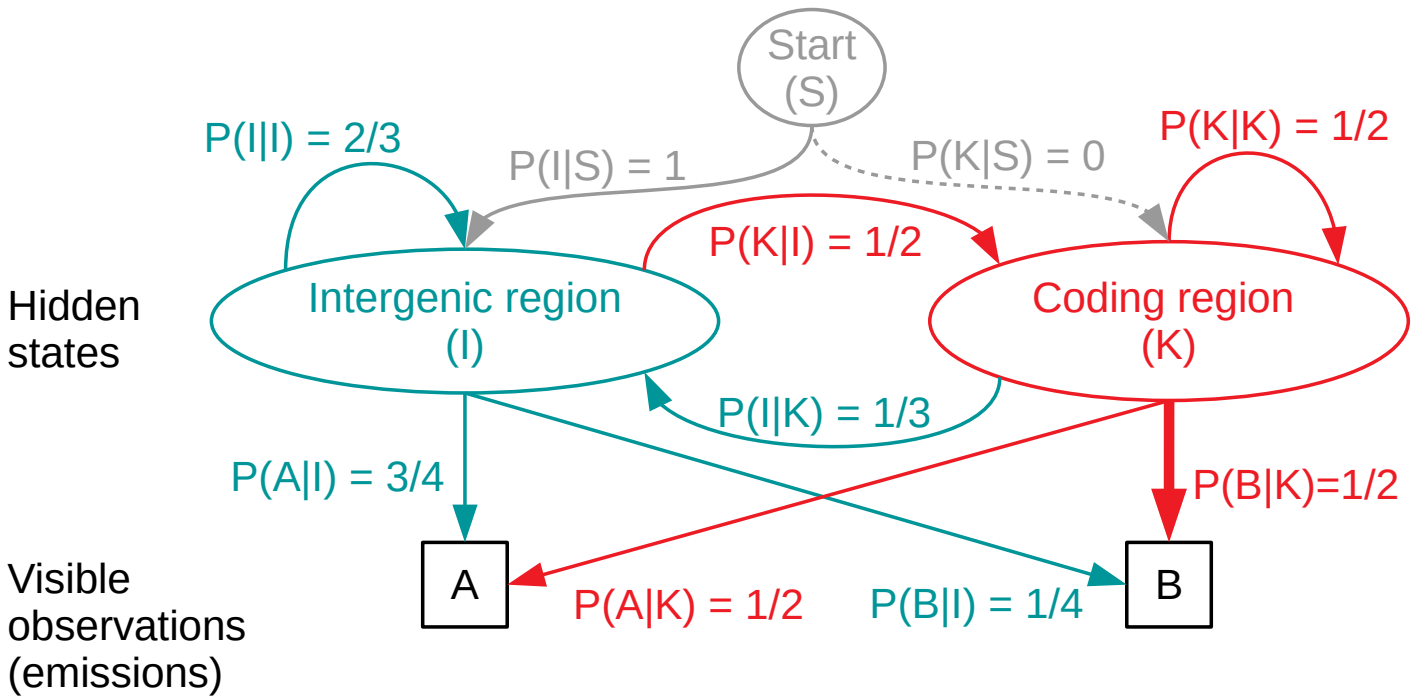
How likely is a given state-emission path?

```
Path = AAB
       IKK

P(Path) = P(I|S)*P(A|I)*P(K|I)*P(A|K)*P(K|K)*P(B|K)
```

Basis of highly accurate gene prediction tools

Hidden Markov Model



How likely is a given state-emission path?

```
Path = AAB
       IKK

P(Path) = P(I|S)*P(A|I)*P(K|I)*P(A|K)*P(K|K)*P(B|K)

        = 1 * ¾ * ½ * ½ * ½ * ½

        = 3/64
```

Find the most probable state sequence for a given sequence

Input: “genome sequence”

AABBBABA

Problem: “too many possible state sequences”

IIIKKKKKK
KKIKKIIIK
IIKIIIKIK
IKKIKIIIK
KIKIKKKIK
KKKIKIKKK
...

Idea:

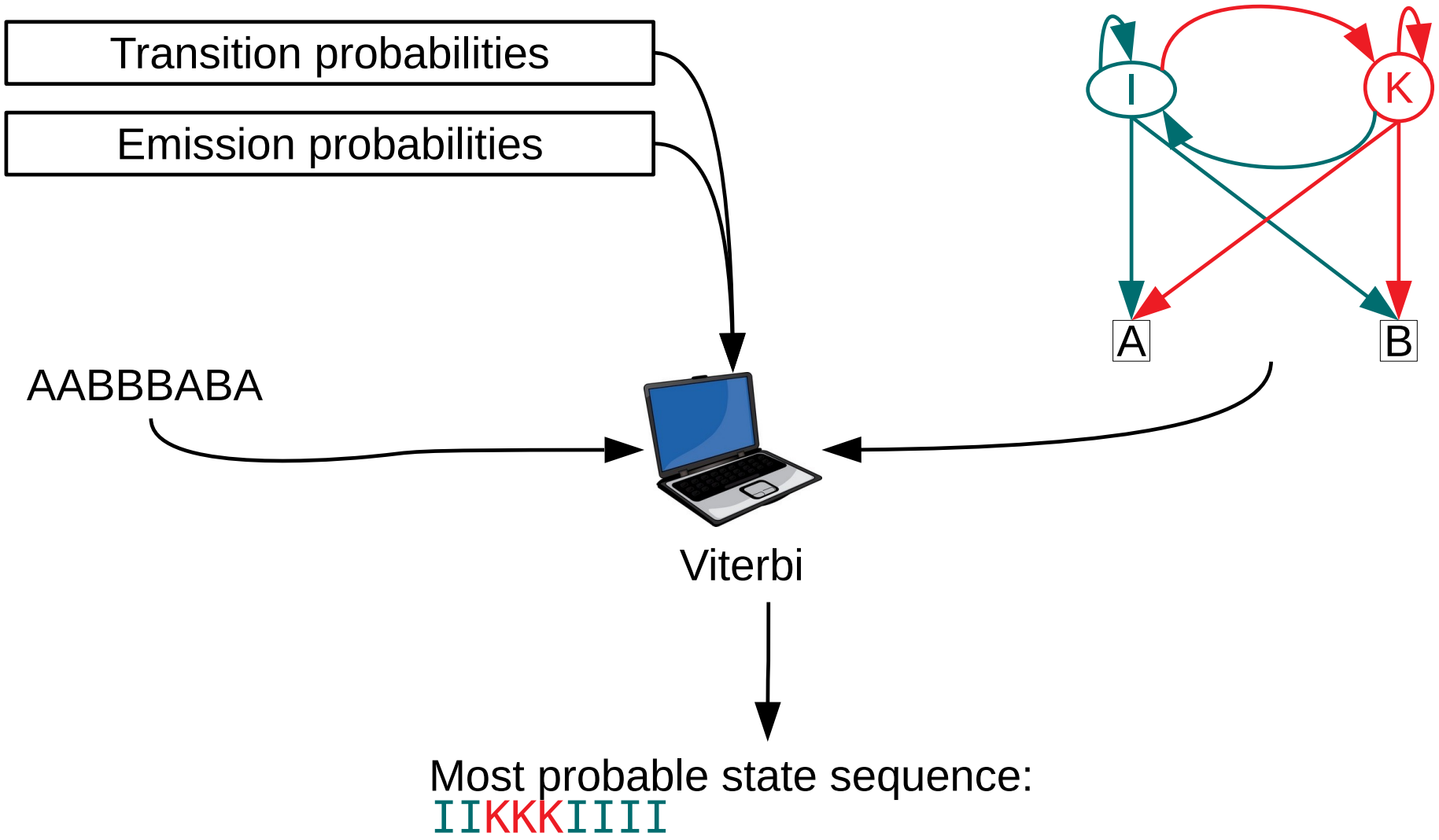
1. Generate all possible state sequences
2. Calculate the probability for each state sequence
3. Choose the state sequence with the highest probability

$\Rightarrow$ too expensive!

Basis of highly accurate gene prediction tools

Hidden Markov Model

Find the most probable state sequence for a sequence: Viterbi Algorithm.



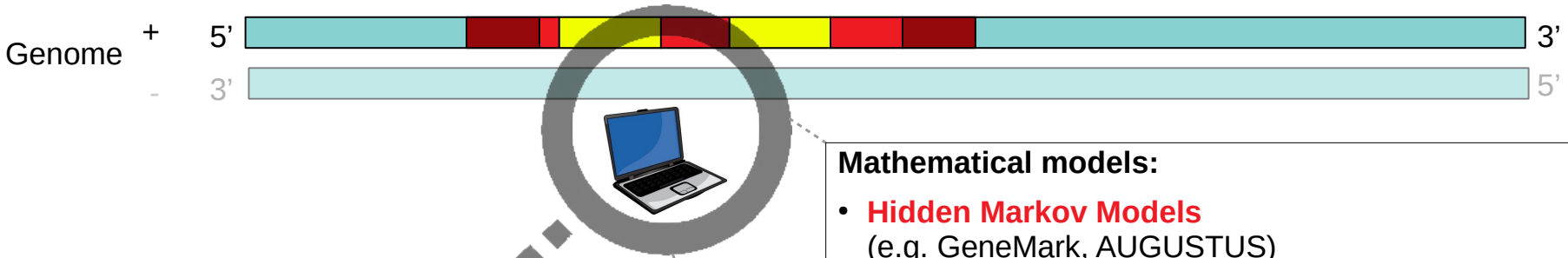
Hidden Markov Model prior Deep Learning in Practice

- 4096 observed nucleotide hexamers
- Many more hidden states
  (e.g. 3'-UTR, 5'-UTR, Intron, ...)

?

Gene
(Parameter training)

Gene
(Prediction)



Transcription data:

- Expressed Sequence Tags
- cDNA
- mRNA-Seq
- mRNA IsoSeq
- ...

Proteome data:

- MS/MS peptides
- proteins of related species
- proteins that every species must have
- ...

Mathematical models:

- **Hidden Markov Models** (e.g. GeneMark, AUGUSTUS)
- dynamic programming
- Support Vector Machines
- neural networks
- decision tree systems
- ...

Provide information on:

- complete gene structures (sometimes incl. UTRs)

Limitations

- *predictions* may be wrong
- models use **parameters** that have to be trained

Many genomes are annotated by database providers

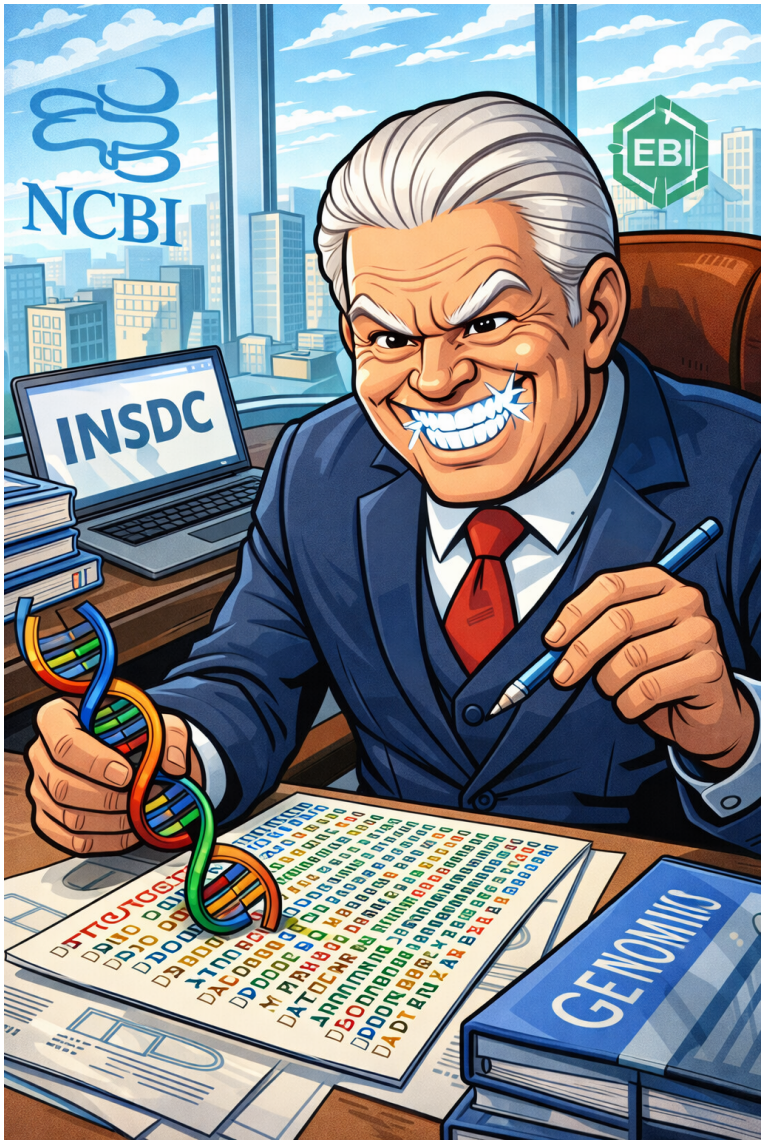
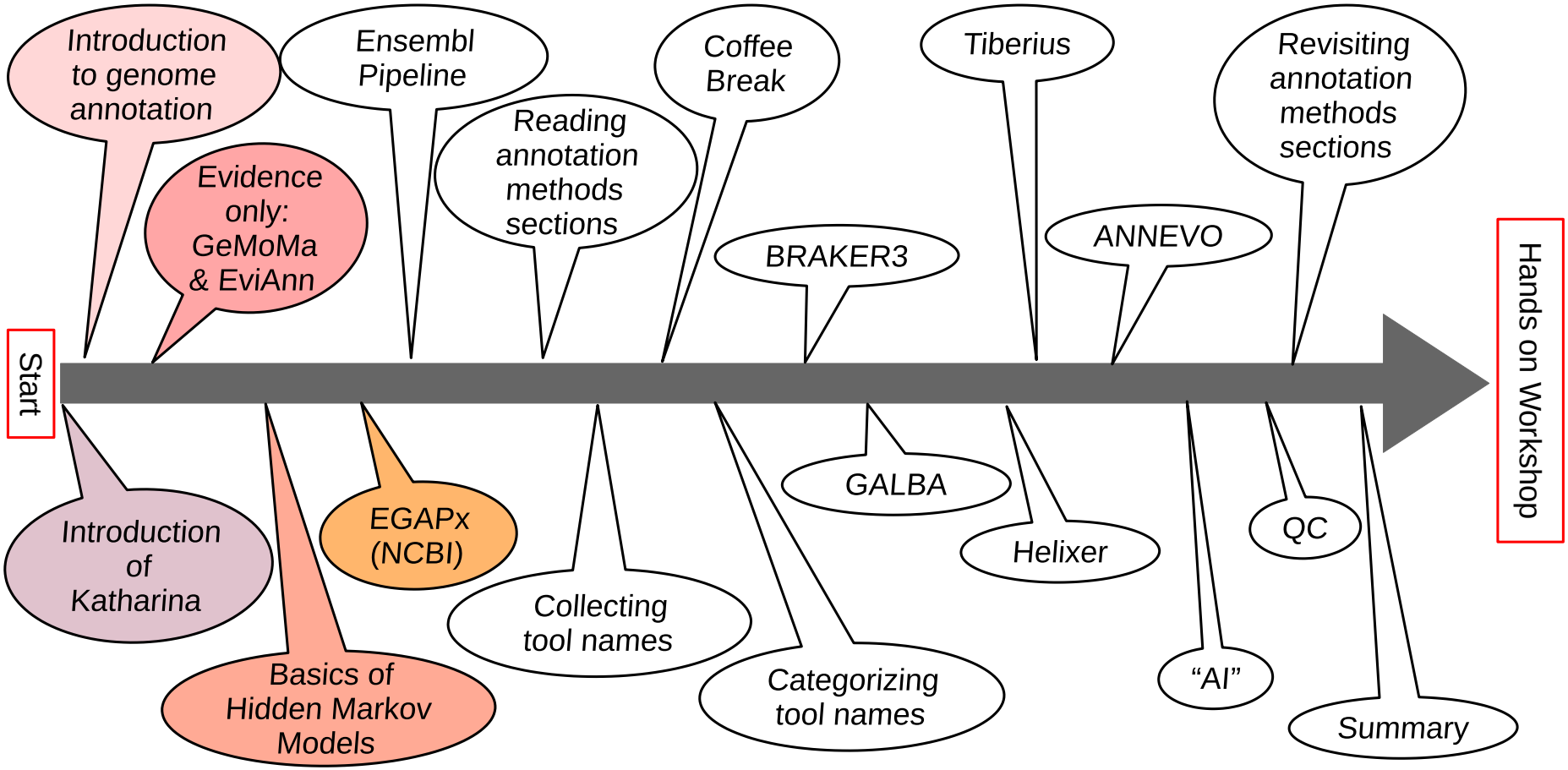


Image: ChatGPT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Tiberius
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Evidence only: GeMoMa & EviAnn
Reading annotation methods sections
BRAKER3
ANNEVO
Start
Hands on Workshop
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EGAPx (NCBI)
GALBA
QC
Helixer
Collecting tool names
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Categorizing tool names
“AI”
Summary

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)

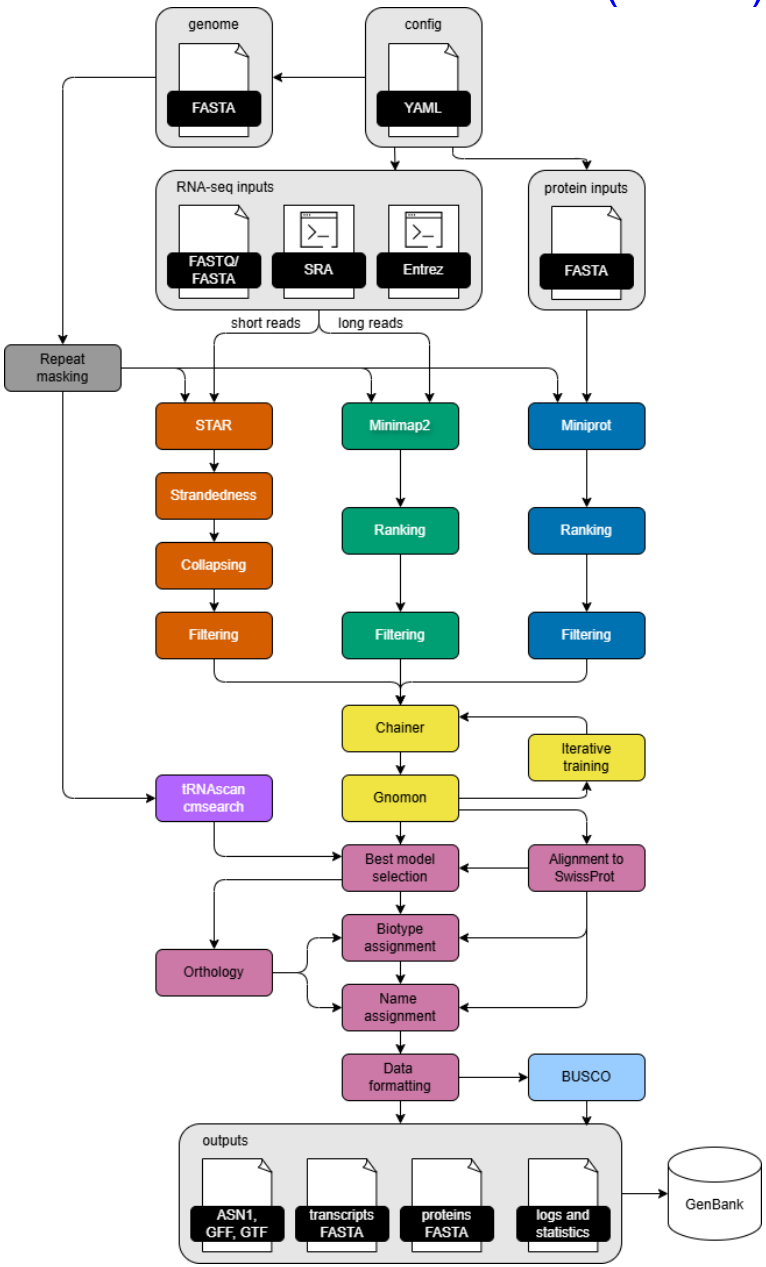
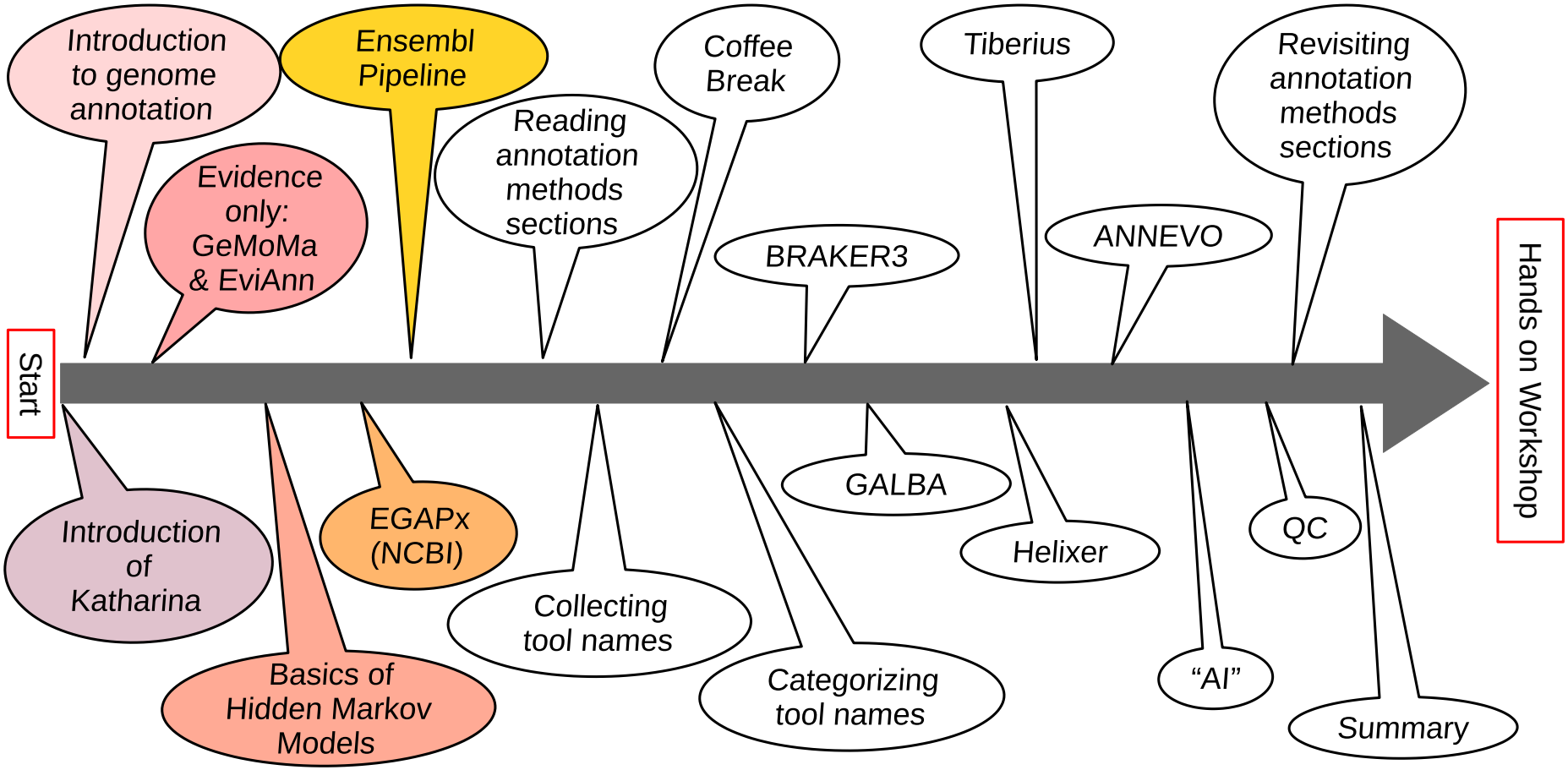


Image: https://github.com/ncbi/egapx

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

Ensembl annotation pipelines

Ensembl annotation pipeline for non-vertebrates

Documentation

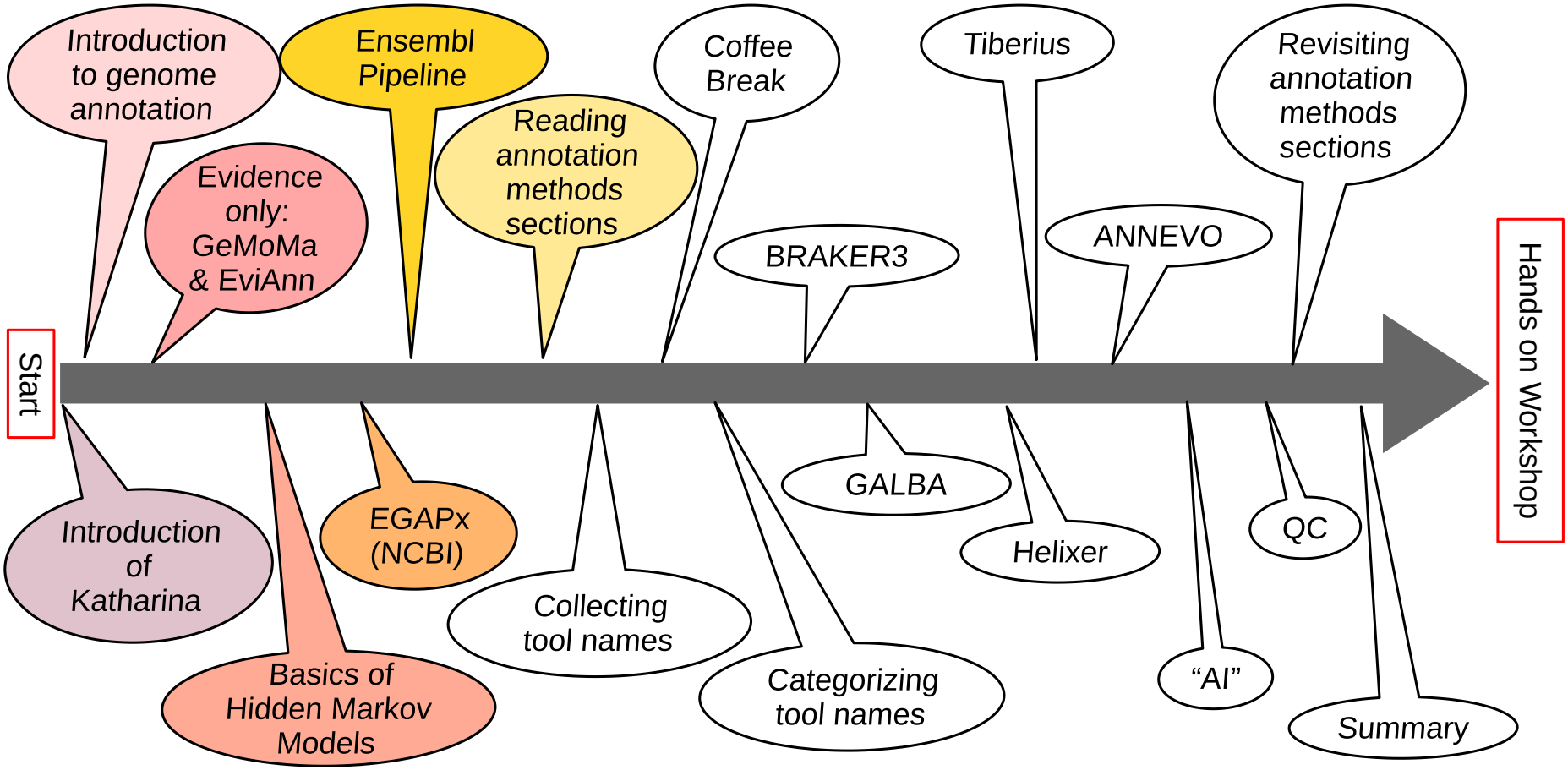
- Ensembl vertebrate pipeline: `https://beta.ensembl.org/help/articles/vertebrate-genome-annotation`
- Ensembl non-vertebrate pipeline: `https://beta.ensembl.org/help/articles/non-vertebrate-genome-annotation`
- BRAKER2 in Ensembl: `https://beta.ensembl.org/help/articles/braker-2-genome-annotation`

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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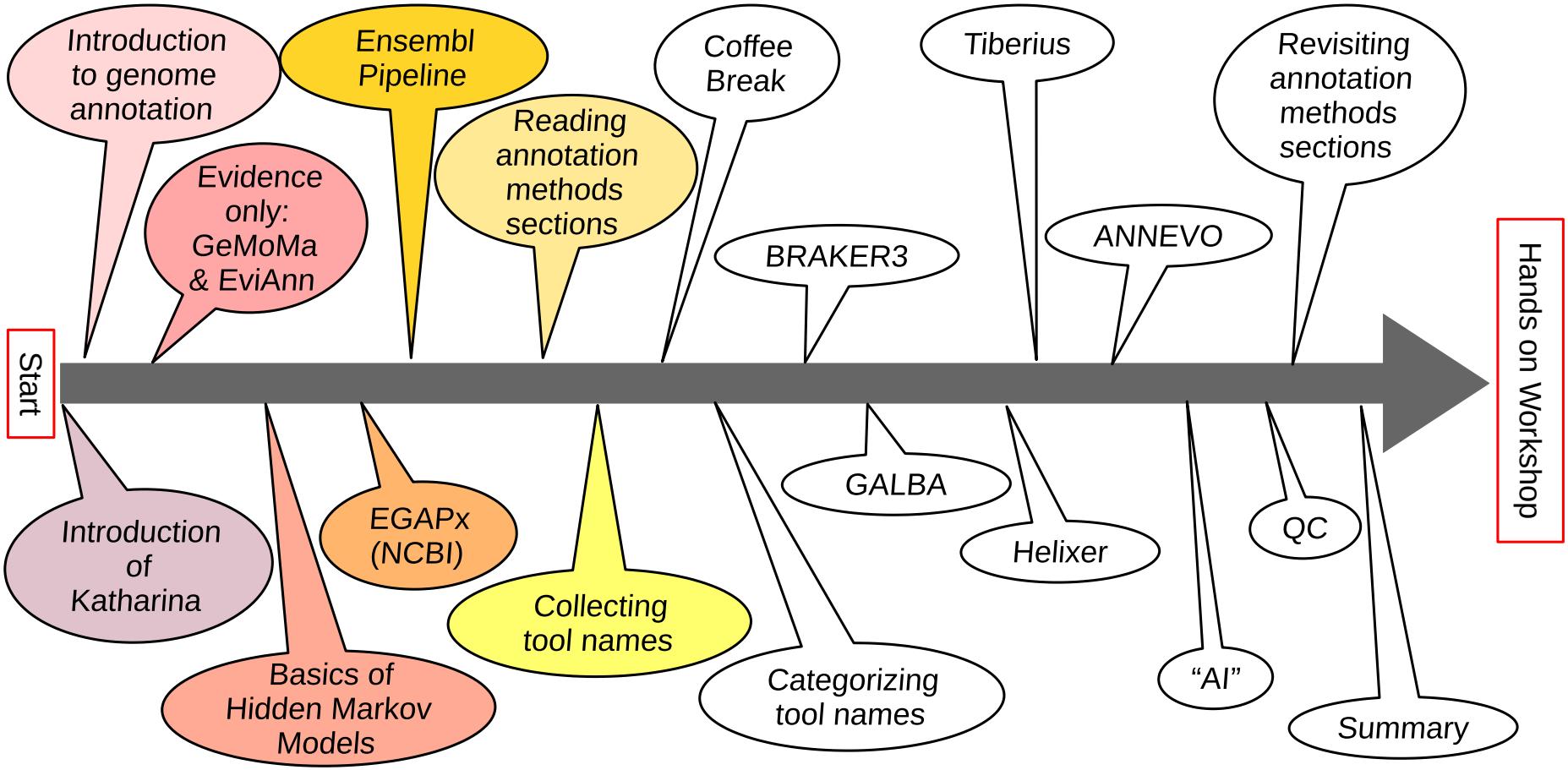
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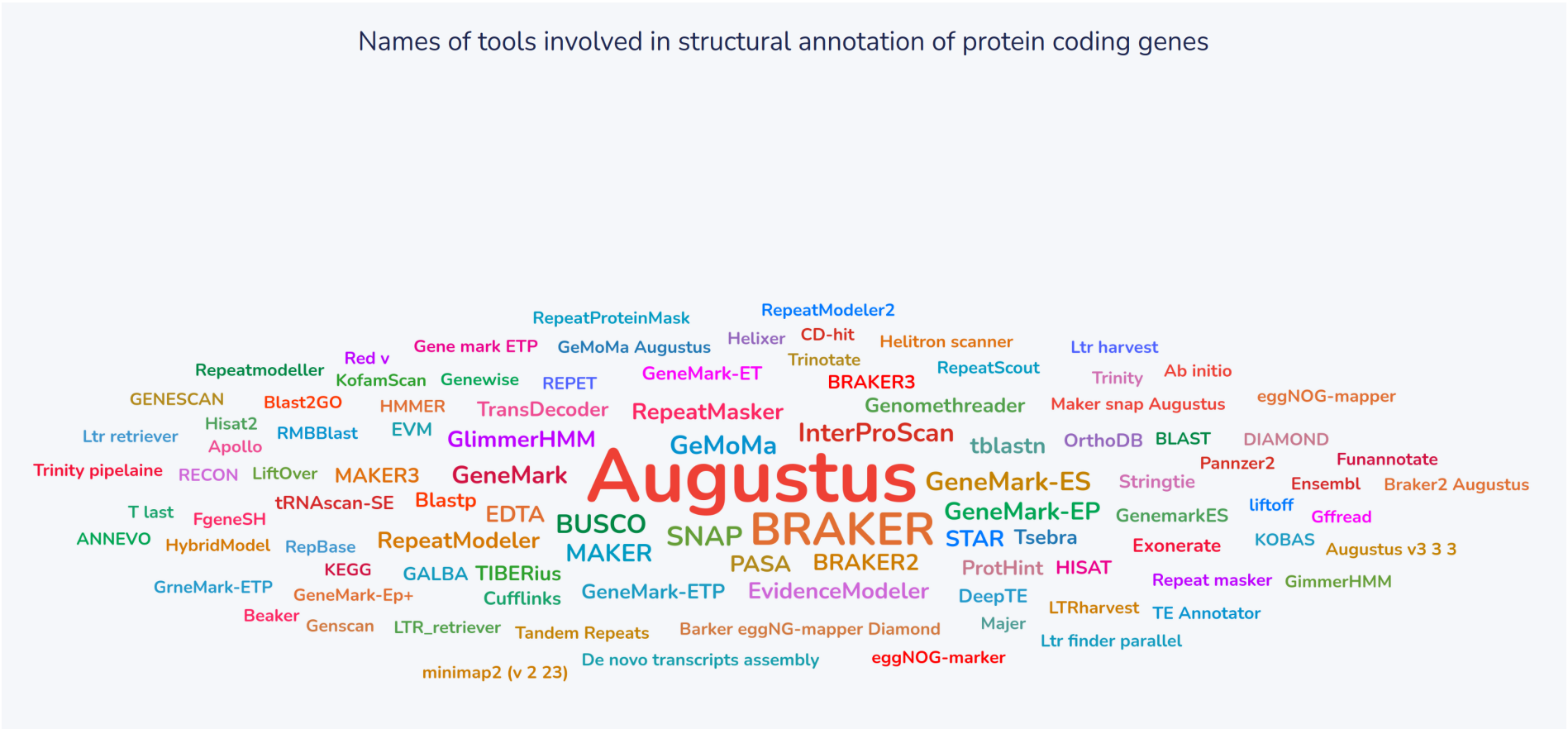
Read your methods snippet

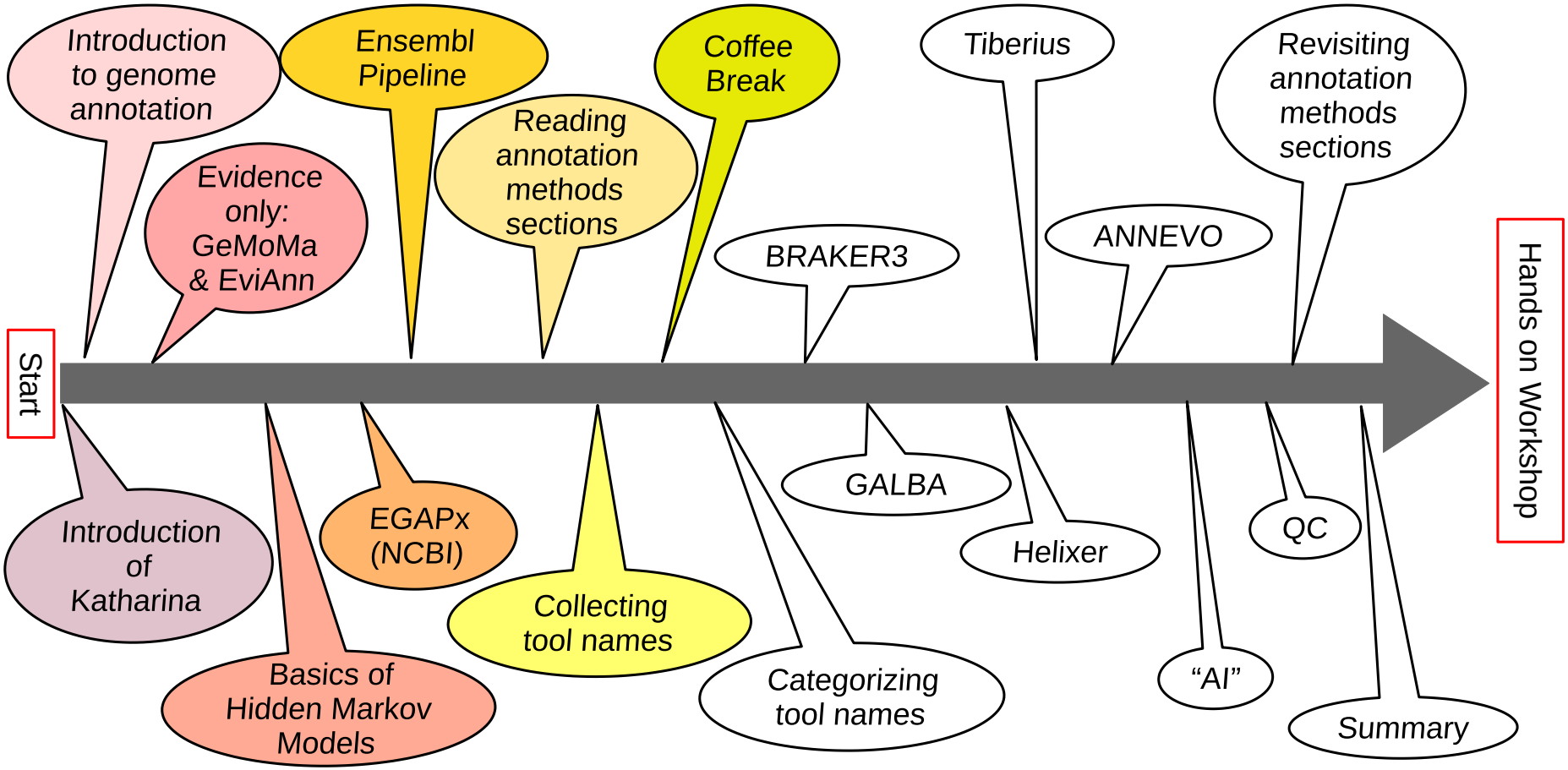
Focus on structural annotation of protein coding genes only!

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

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Tiberius
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Evidence only: GeMoMa & EviAnn
Reading annotation methods sections
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ANNEVO
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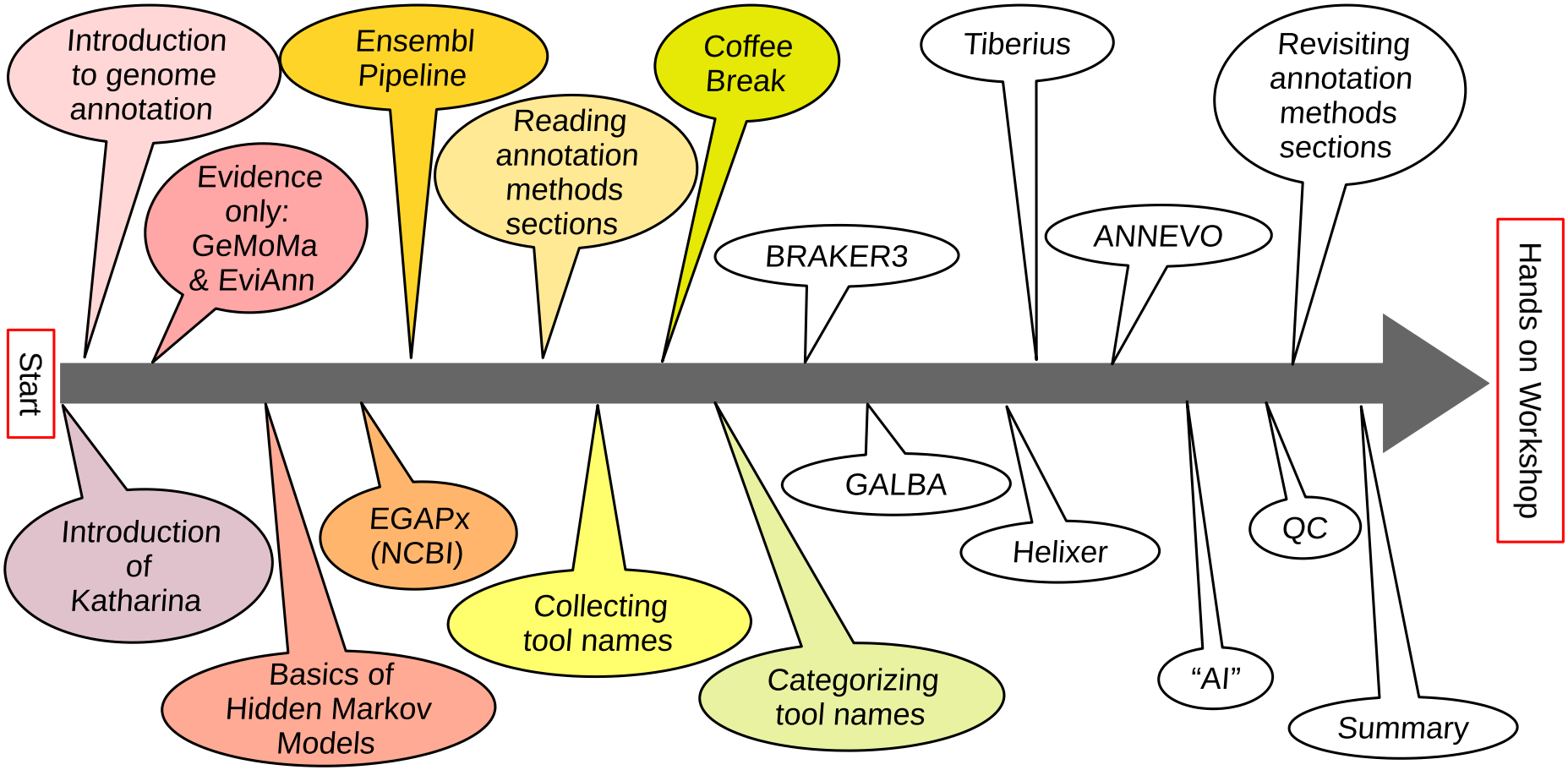
Hands on Workshop
Introduction of Katharina
EGAPx (NCBI)
GALBA
QC
Helixer
Collecting tool names
Basics of Hidden Markov Models
Categorizing tool names
“AI”
Summary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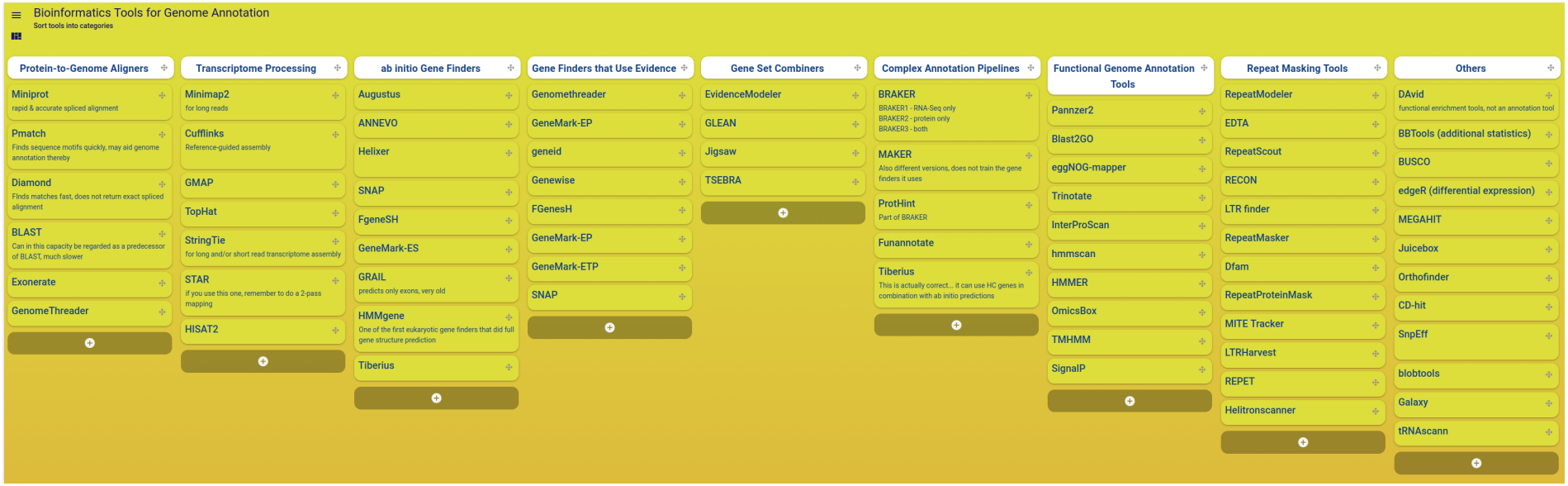
Categorize tool names

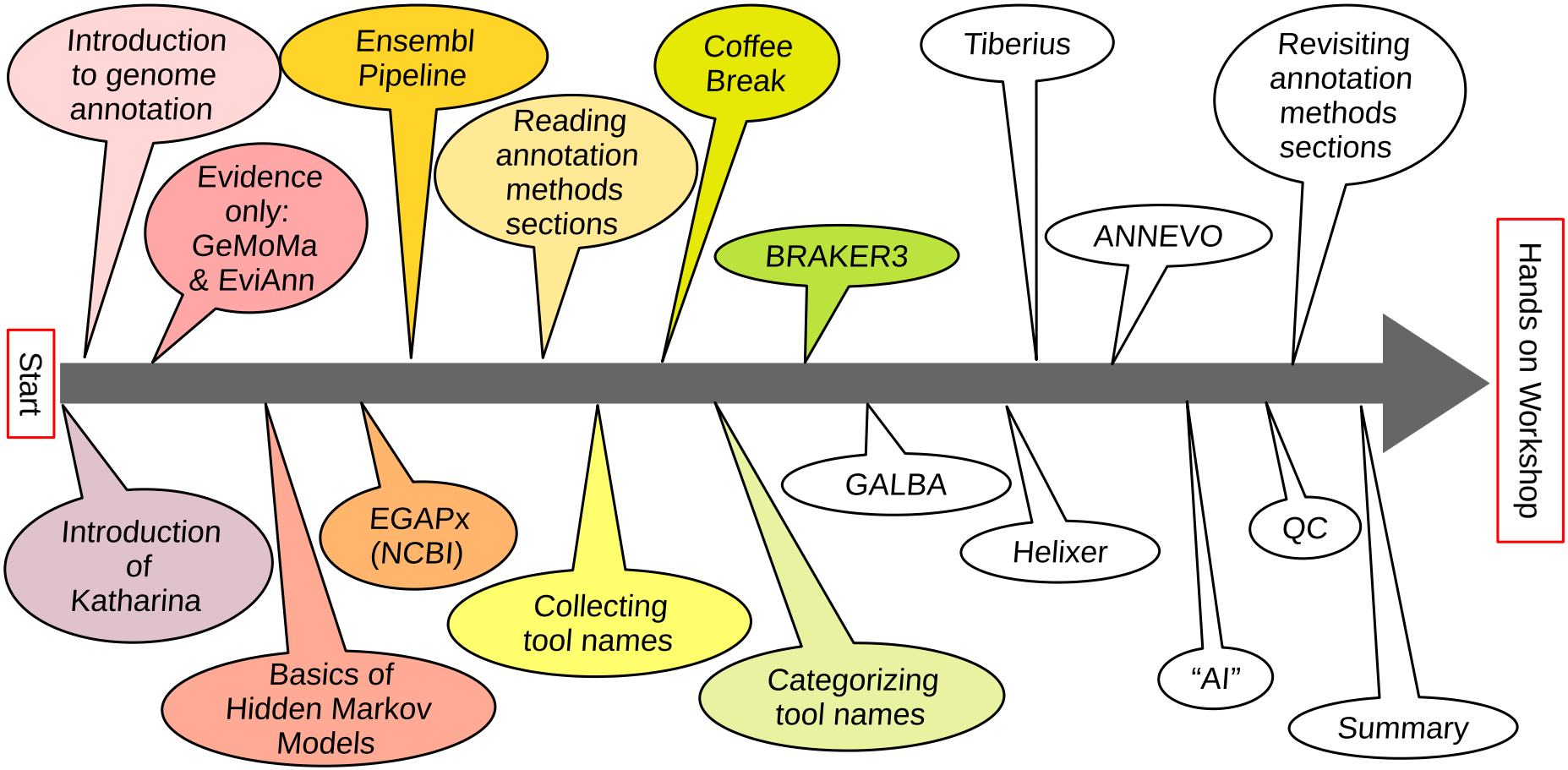
Go to

`https://shorturl.at/uA0Tg`

and sort the tools names from your methods snippet into categories

Categorize tool names

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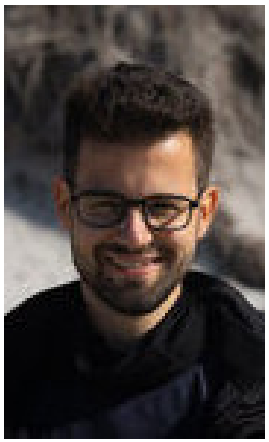
Genome annotation super heros enable the community

Image: ChatGPT

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

Historical context on BRAKER

Once upon a time...

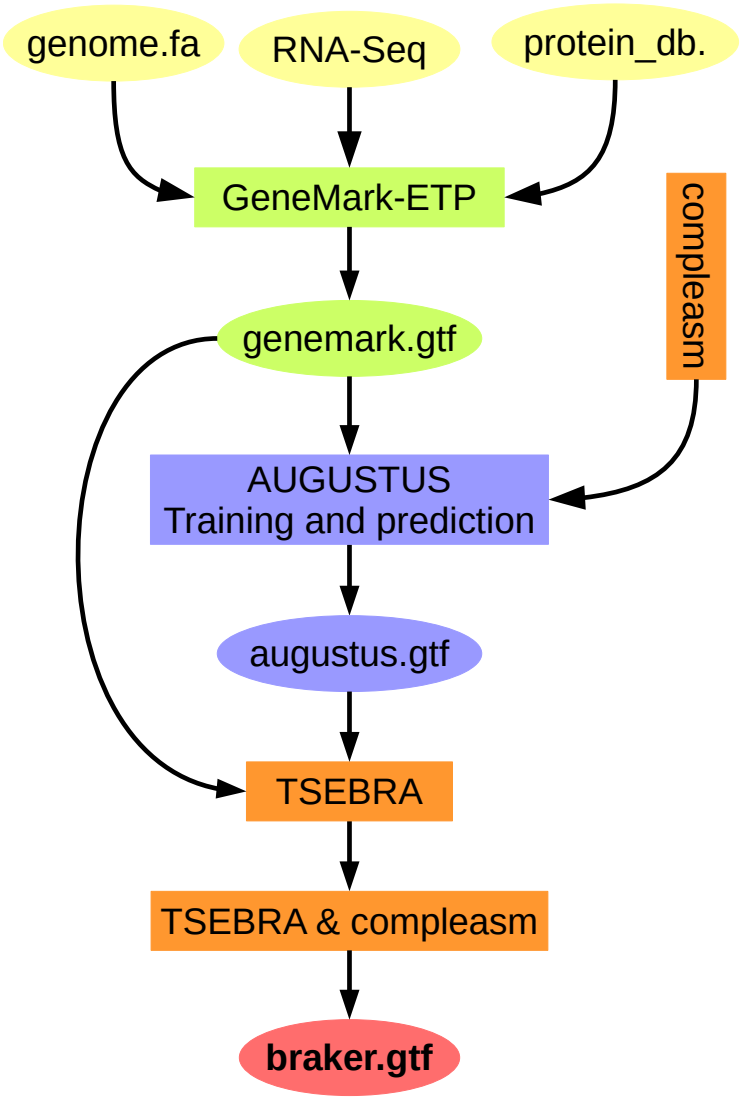
- AUGUSTUS requires supervised training & predicts alternative isoforms
- GeneMark performs unsupervised training
- MAKER integrated both tools for inference, not for training
- BRAKER1: GeneMark-ET + AUGUSTUS + RNA-Seq
- BRAKER2: GeneMark-EP + AUGUSTUS + proteins
- BRAKER3: GeneMark-ETP + AUGUSTUS + RNA-Seq + proteins

... but it is all one pipeline: `braker.pl`

Reasons for making BRAKER

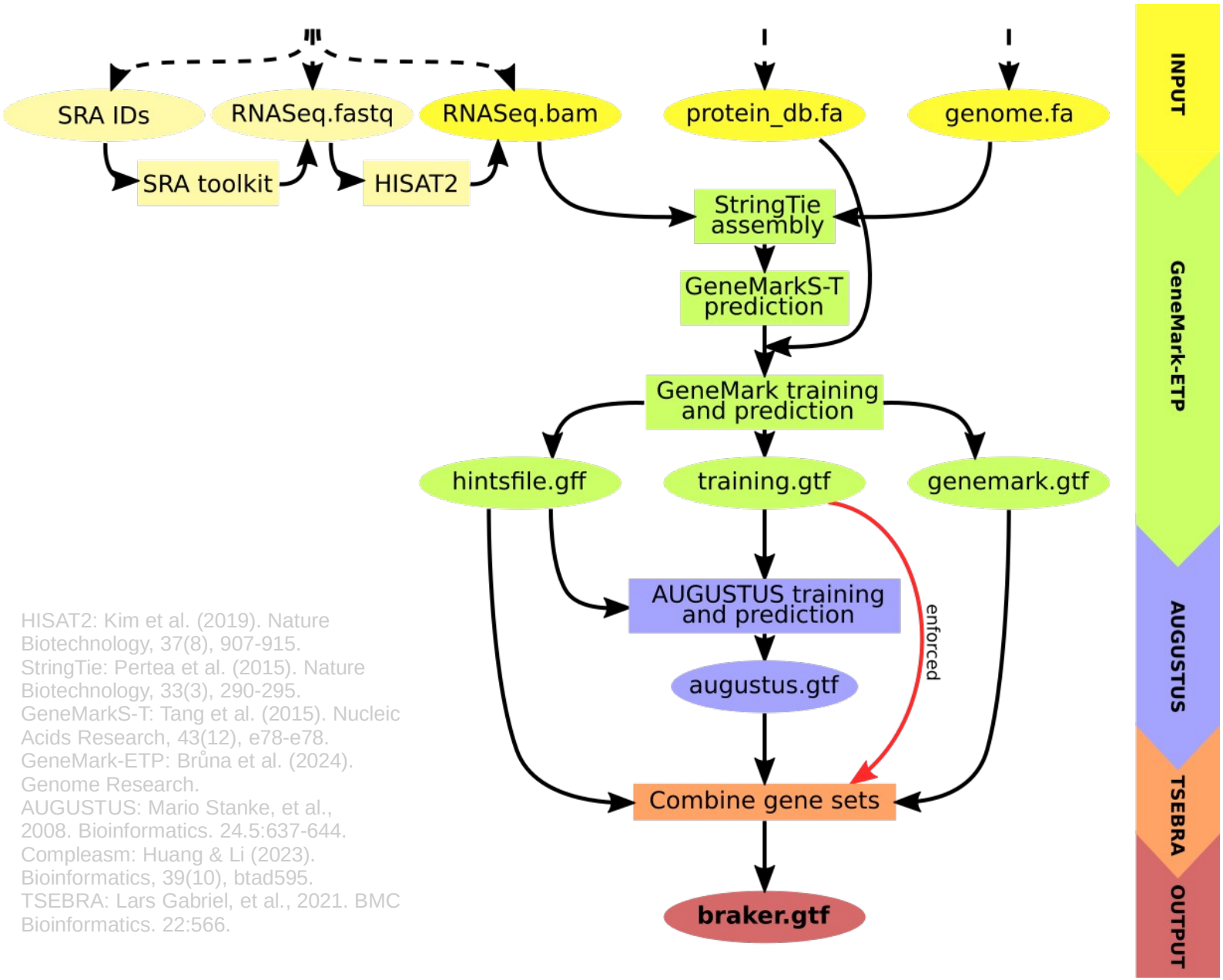
- increasing accuracy
- providing automation
- restoring citations

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



- Gabriel *et al.* (2024)
- 4,371 citations (all BRAKER publications, 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



HISAT2: Kim et al. (2019). Nature Biotechnology, 37(8), 907-915.
StringTie: Pertea et al. (2015). Nature Biotechnology, 33(3), 290-295.
GeneMarkS-T: Tang et al. (2015). Nucleic Acids Research, 43(12), e78-e78.
GeneMark-ETP: Brůna et al. (2024). Genome Research.
AUGUSTUS: Mario Stanke, et al., 2008. Bioinformatics. 24.5:637-644.
Compleasm: Huang & Li (2023). Bioinformatics, 39(10), btad595.
TSEBRA: Lars Gabriel, et al., 2021. BMC Bioinformatics. 22:566.

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

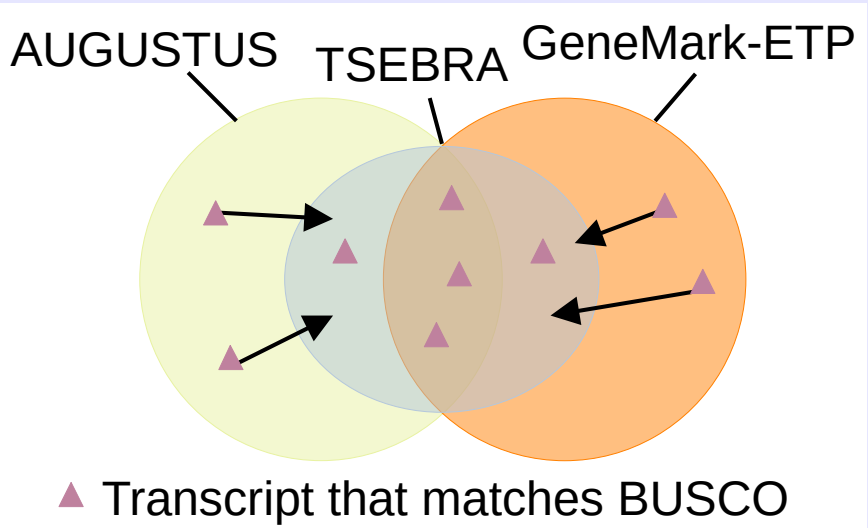
SOFTWARE Open Access

TSEBRA: transcript selector for BRAKER

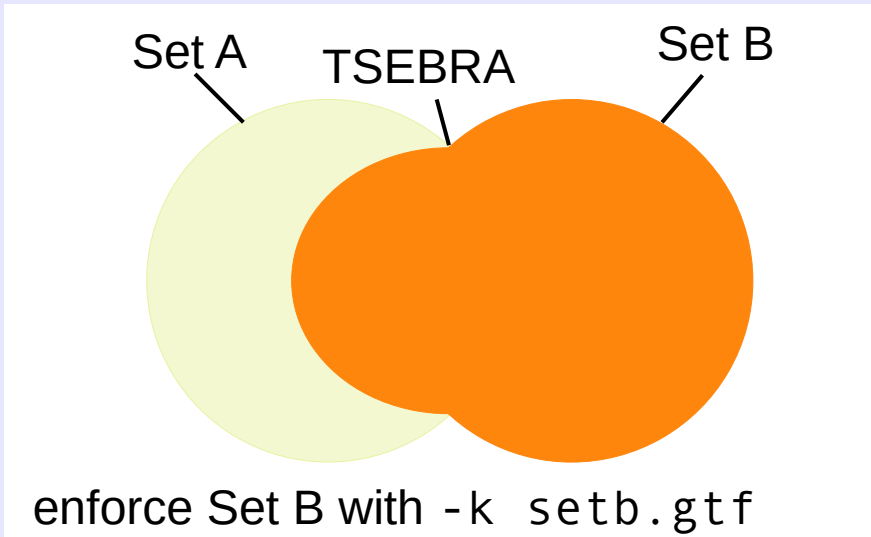
Lars Gabriel[1,2], Katharina J. Hoff[1,2], Tomáš Brůna[3], Mark Borodovsky[4,5] and Mario Stanke[1,2*]

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

TSEBRA in BRAKER



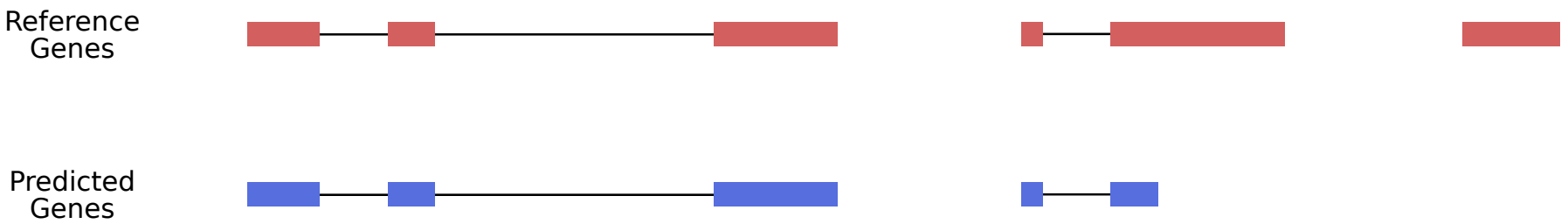
Manually enforcing a gene set



Is also used in Tiberius evidence processing pipeline.

Measuring accuracy of genome annotation

Compare prediction against a high quality reference.

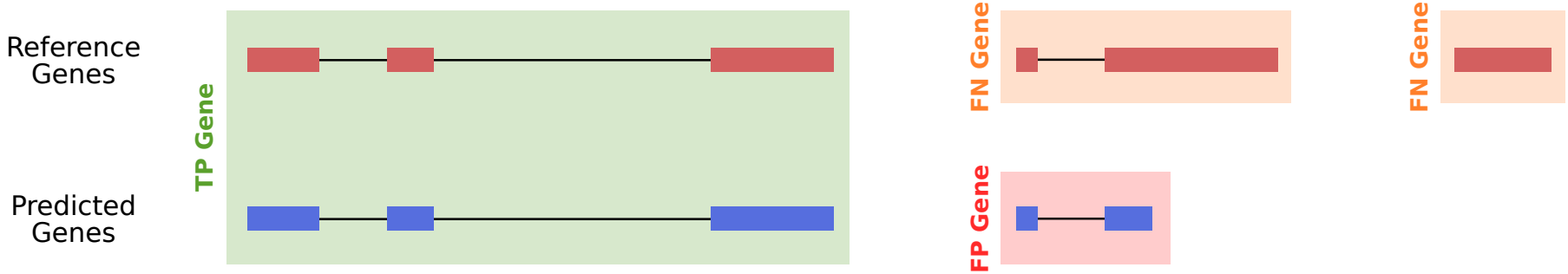


We only look at CDS exons, ignoring UTRs.

Measuring accuracy of genome annotation

Gene Level Accuracy

TP = True Positive
FP = False Positive
FN = False Negative



$$\text{Sensitivity (Sn)} = \frac{\#\text{TP Genes}}{\#\text{TP Genes} + \#\text{FN Genes}} \qquad \text{Precision (Pr)} = \frac{\#\text{TP Genes}}{\#\text{TP Genes} + \#\text{FP Genes}} \qquad \text{F1-Score} = \frac{2 \cdot \text{Sn} \cdot \text{Pr}}{\text{Sn} + \text{Pr}}$$

We only look at CDS exons, ignoring UTRs.

Accuracy of genome annotation approaches by BRAKER team

	Tools	Evidence
BRAKER1	GeneMark-ET + AUGUSTUS	RNA-Seq
BRAKER2	GeneMark-EP + AUGUSTUS	proteins
BRAKER3	GeneMark-ETP + AUGUSTUS	RNA-Seq + proteins
TSEBRA	BRAKER1 + BRAKER2	RNA-Seq + proteins
GeneMark-ETP		RNA-Seq + proteins

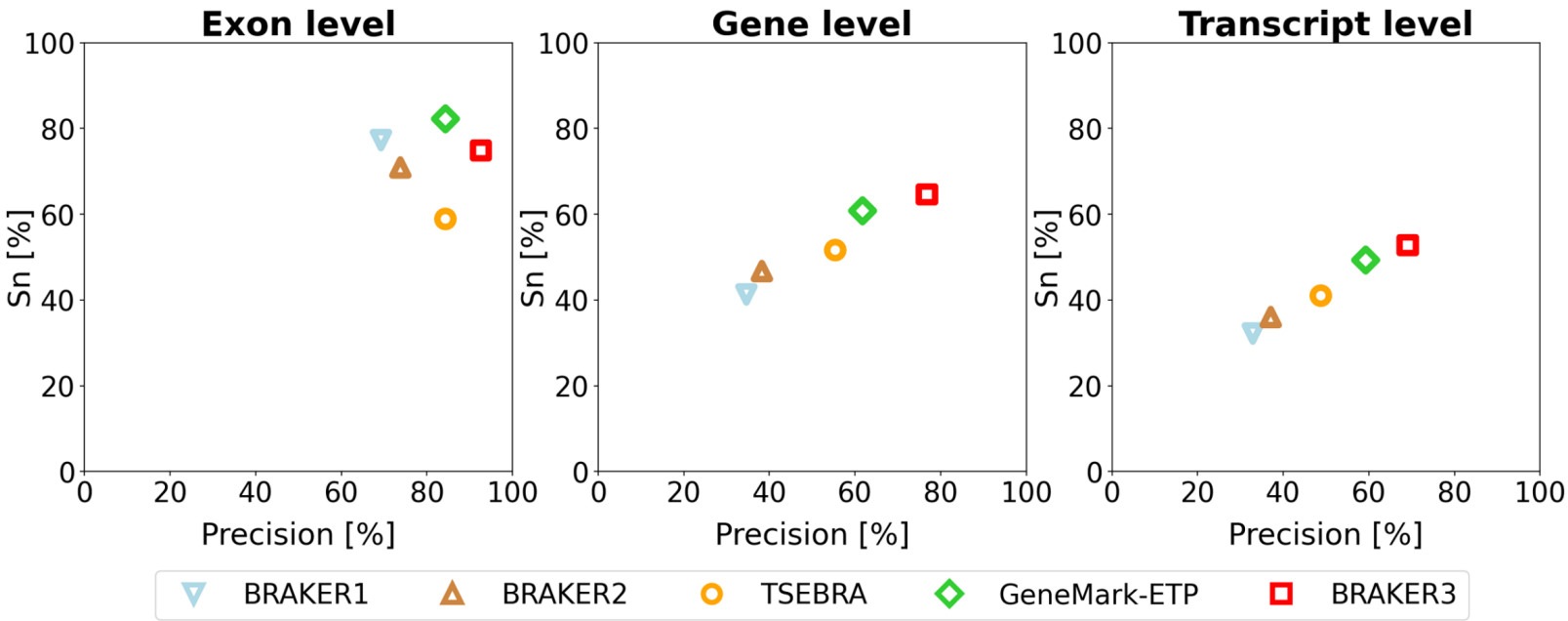


Figure 2. Average precision and sensitivity of gene predictions made by BRAKER1, BRAKER2, TSEBRA, GeneMark-ETP, and BRAKER3 for the genomes of 11 different species (listed in Supplemental Table S1). Inputs were the genomic sequences, short-read RNA-seq libraries, and protein databases (*order excluded*).

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

Availability

GitHub

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

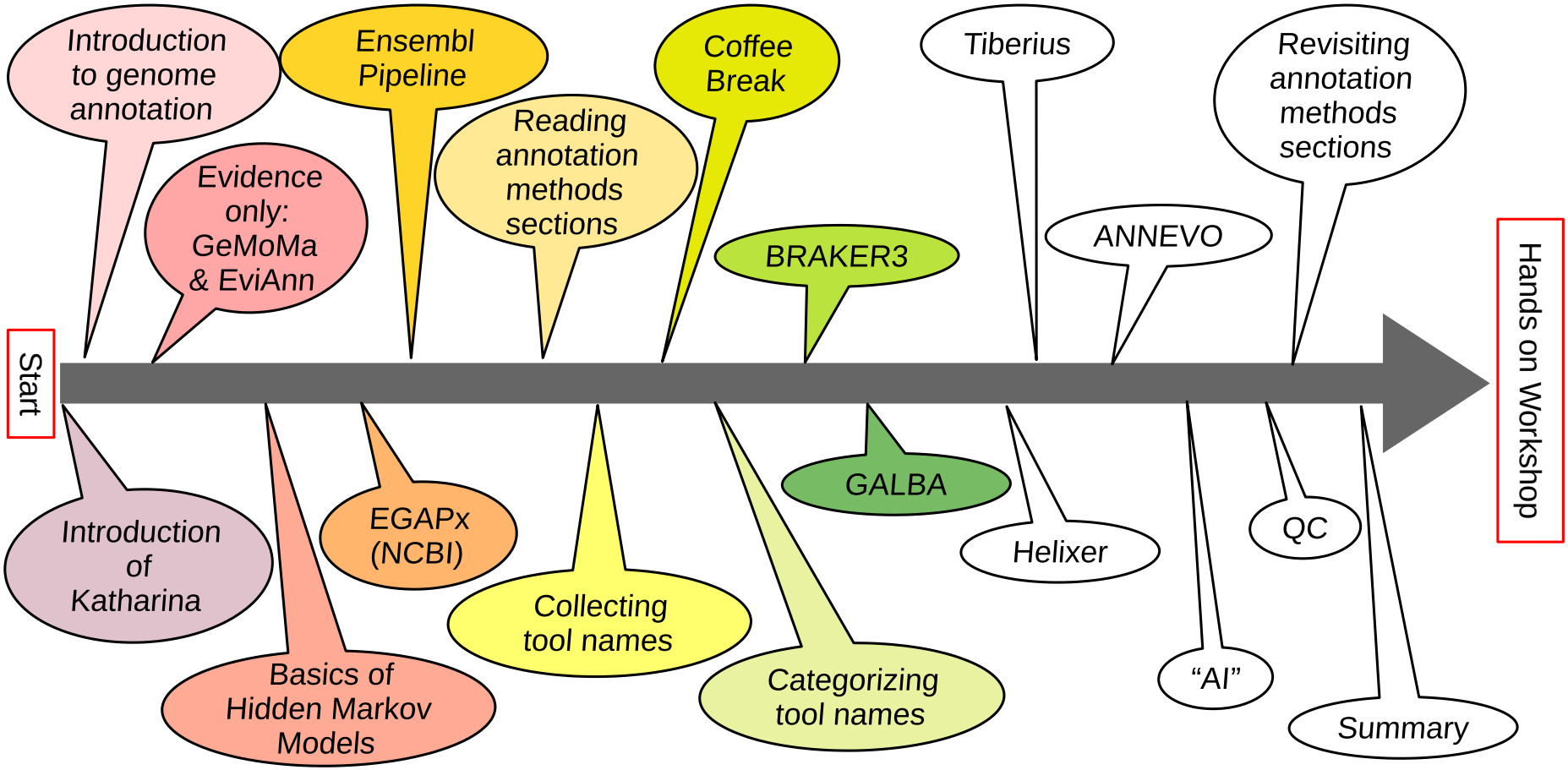
Docker/Singularity

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

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

Licenses

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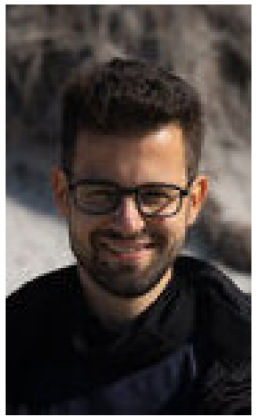
GALBA Contributors

Tomáš Brůna

Heng Li

Joseph Guhlin



Lars Gabriel

Natalia Nenasheva

Ethan Tolman

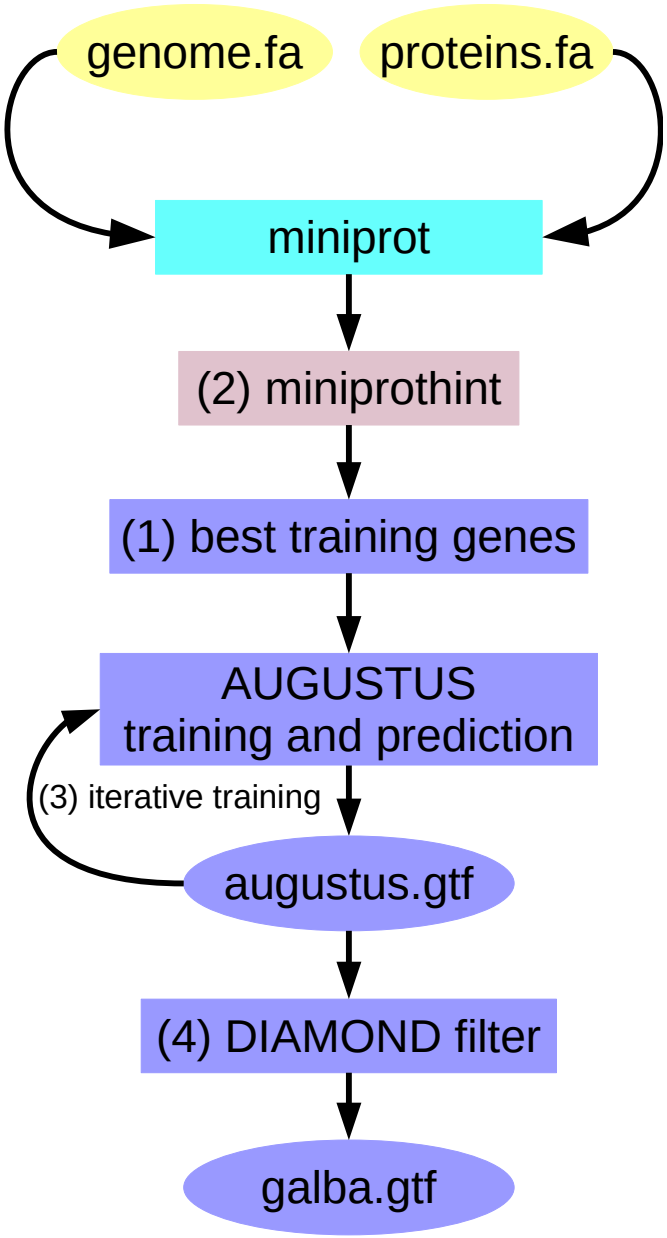
Paul Frandsen

Matthis Ebel

Mario Stanke

Katharina Hoff

Brůna *et al. BMC Bioinformatics* (2023) 24:327
https://doi.org/10.1186/s12859-023-05449-z

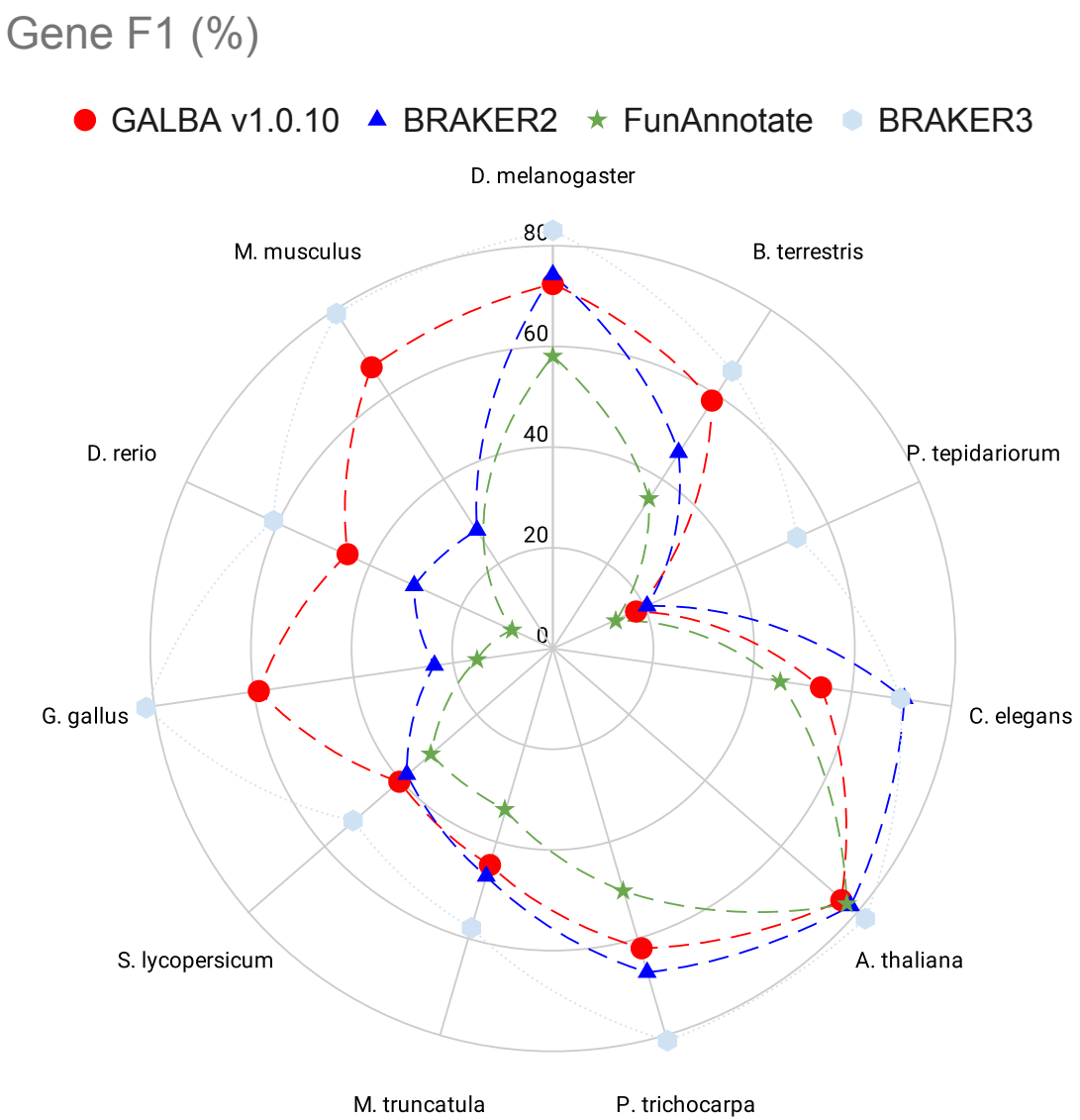


RESEARCH Open Access

Galba: genome annotation with miniprot and AUGUSTUS

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

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



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]
```

Licenses

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

Interlude: EASEL can annotate genomes with very large introns

Image: ChatGPT by OpenAI

EASEL: https://gitlab.com/PlantGenomicsLab/easel

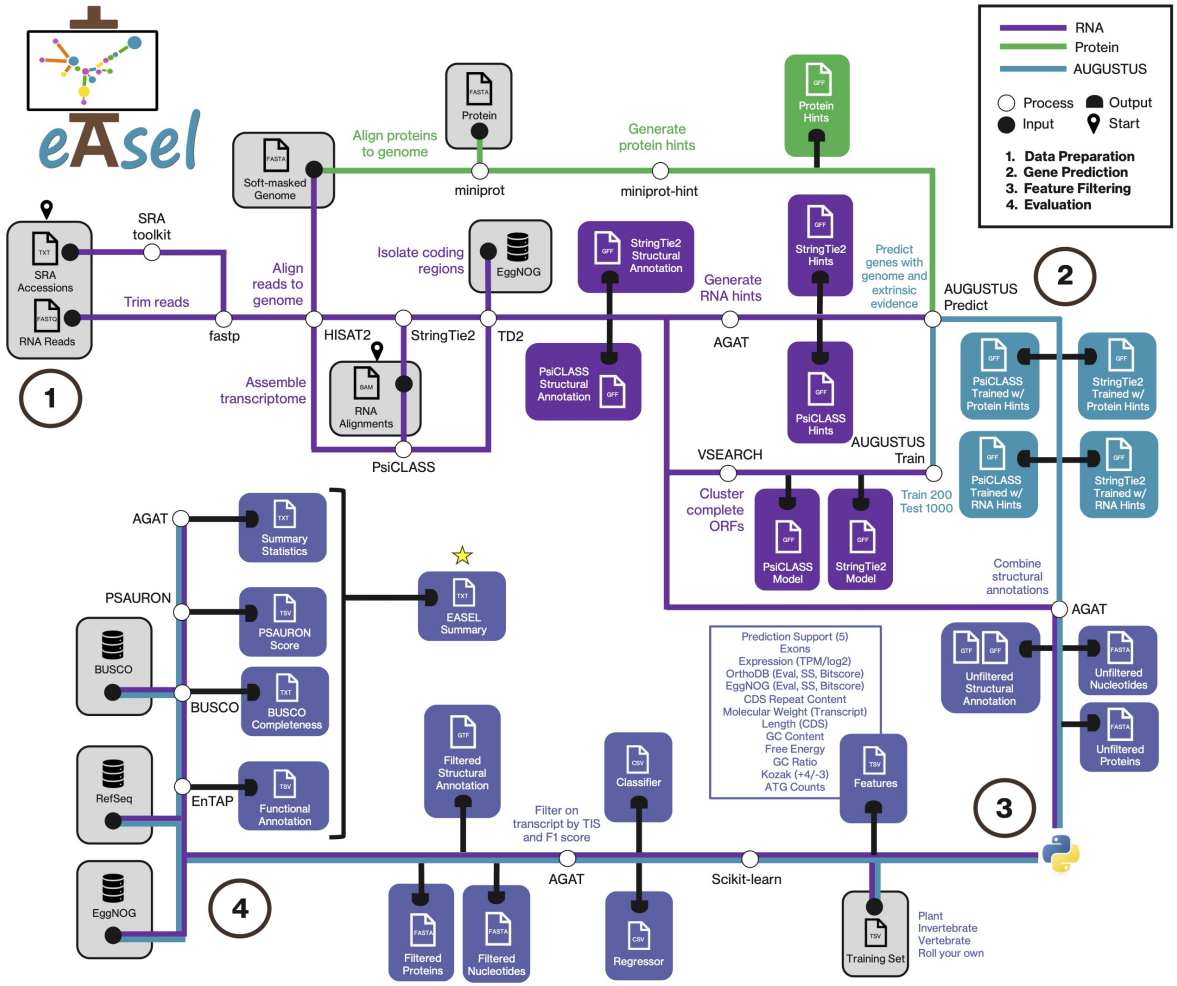
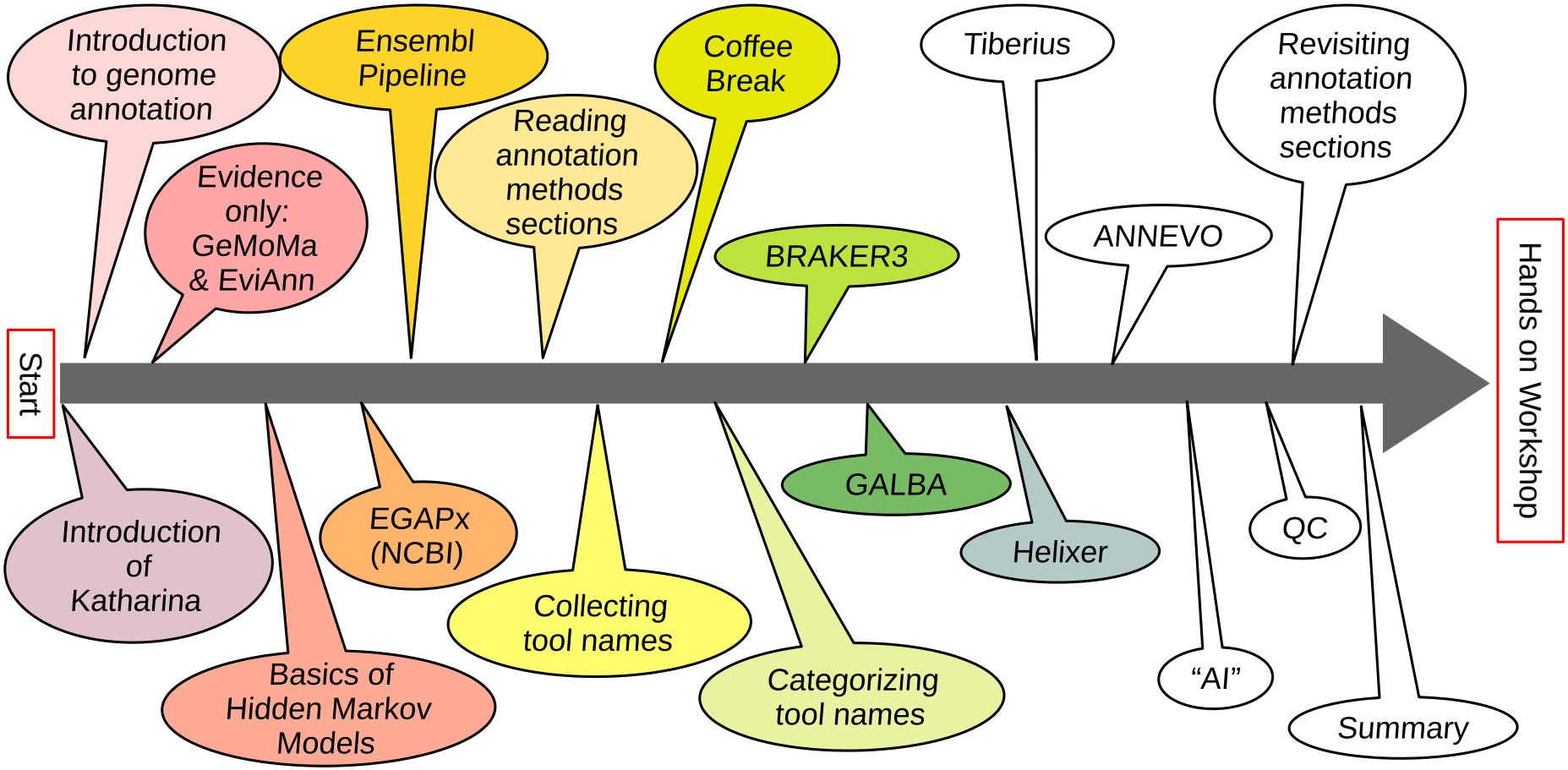


Image: courtesy of Cynthia Webster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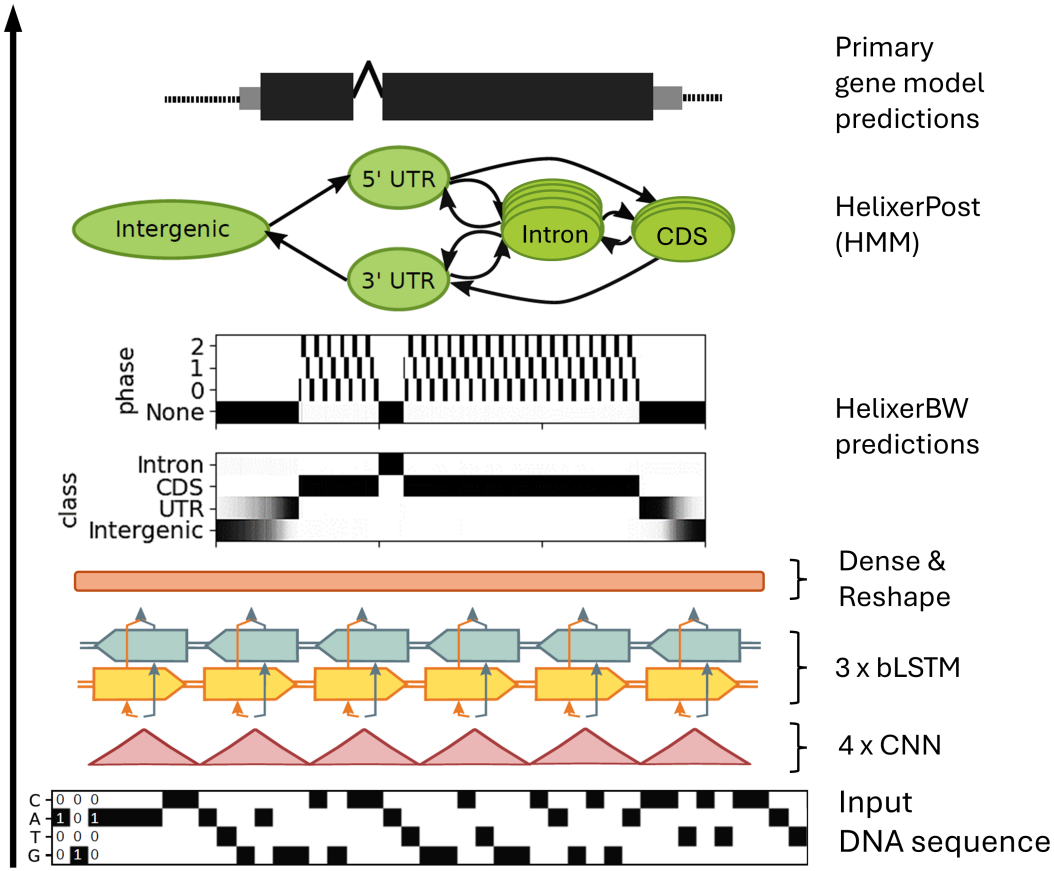
Helixer: bringing deep learning into genome annotation

Image: ChatGPT by OpenAI, manual editing

Article | Open access | Published: 24 November 2025

Helixer: ab initio prediction of primary eukaryotic gene models combining deep learning and a hidden Markov model

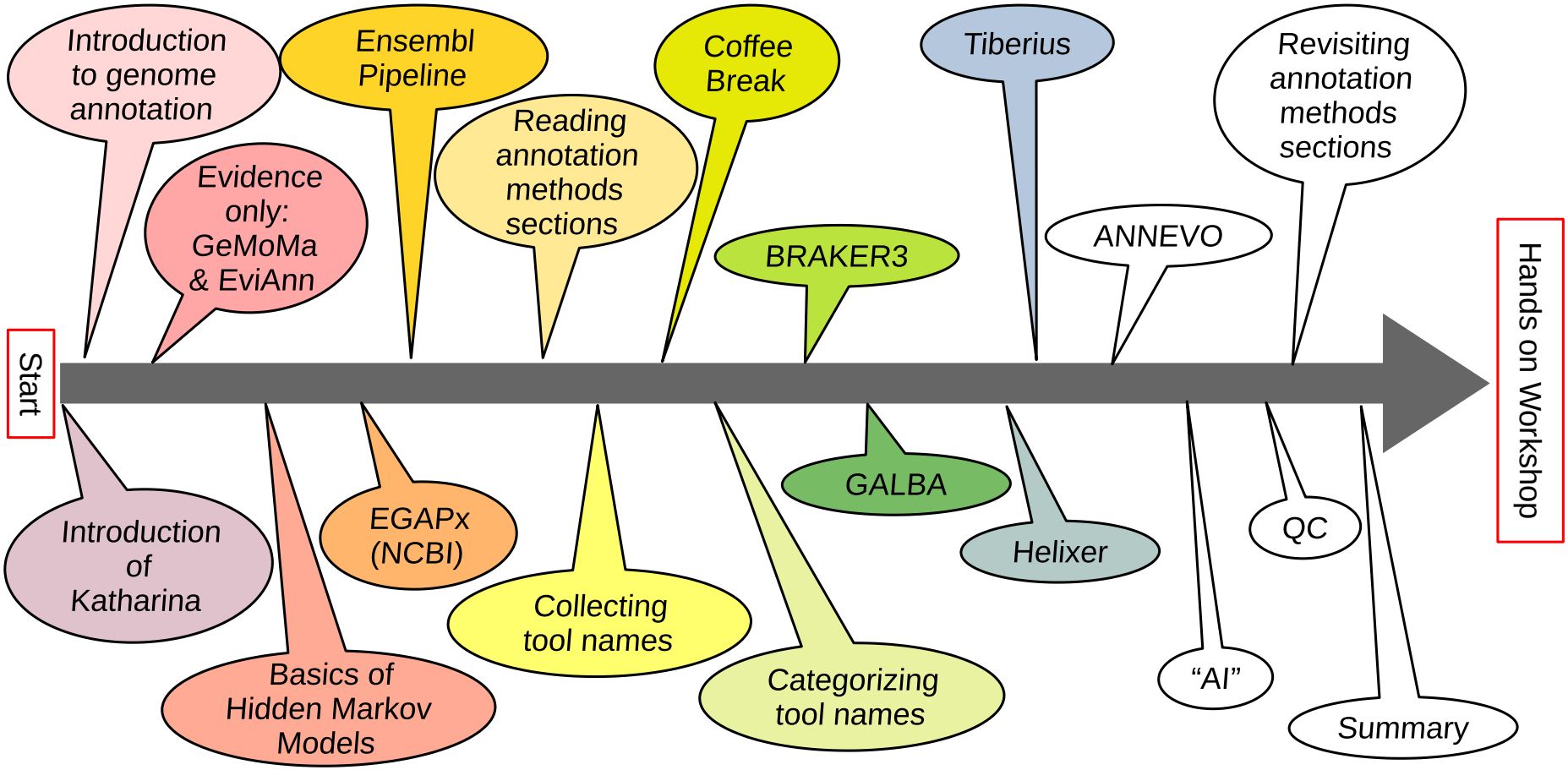
Felix Holst, Anthony M. Bolger, Felicitas Kindel, Christopher Günther, Janina Maß, Sebastian Triesch, Niklas Kiel, Nima Saadat, Oliver Ebenhöh, Björn Usadel, Rainer Schwacke, Andreas P. M. Weber, Marie E. Bolger ✉ & Alisandra K. Denton



- 206 citations (all pubs, 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`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Tiberius: improved genome annotation with deep learning

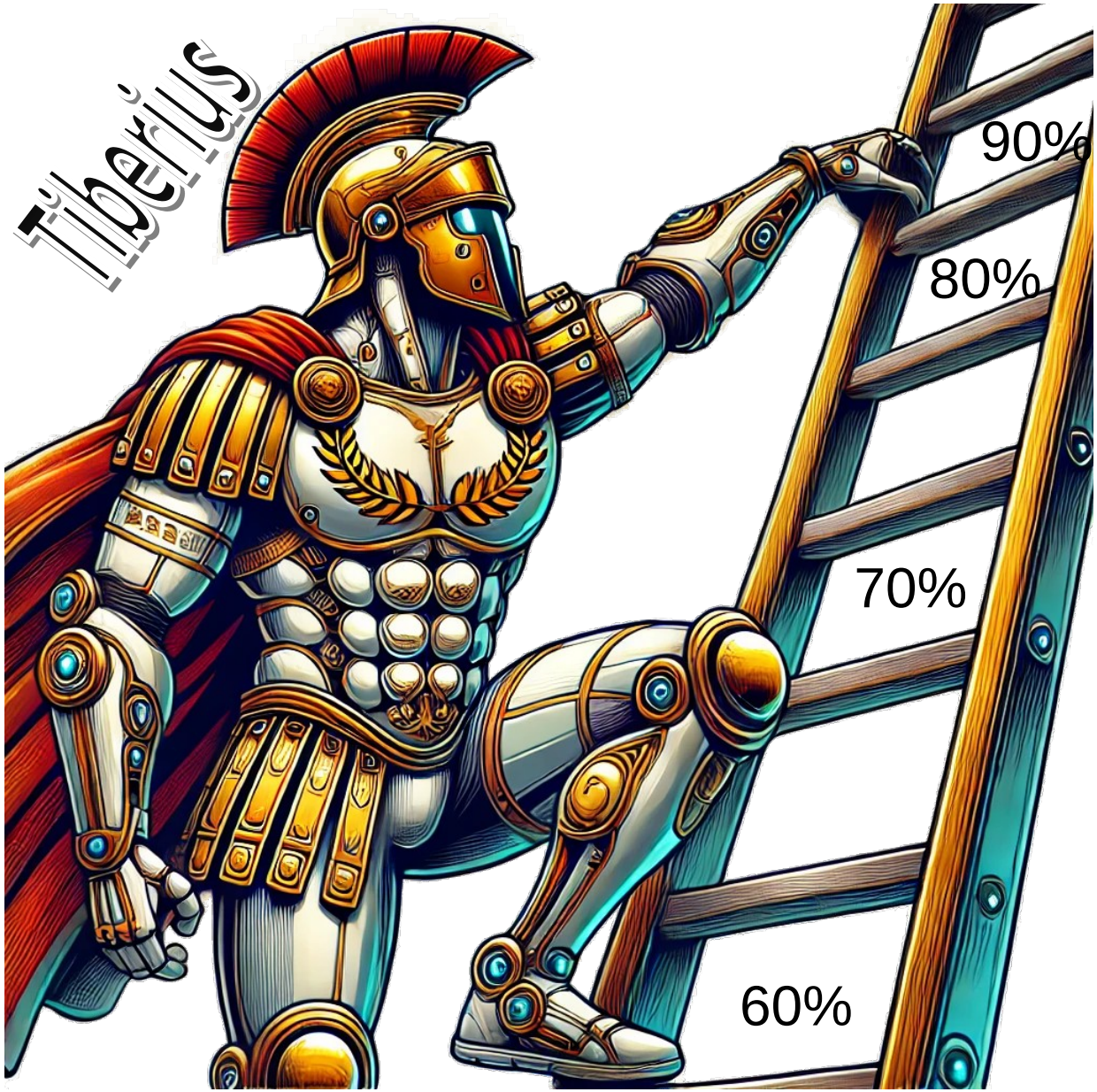


Image: ChatGPT by OpenAI, manual editing

The Tiberius Team

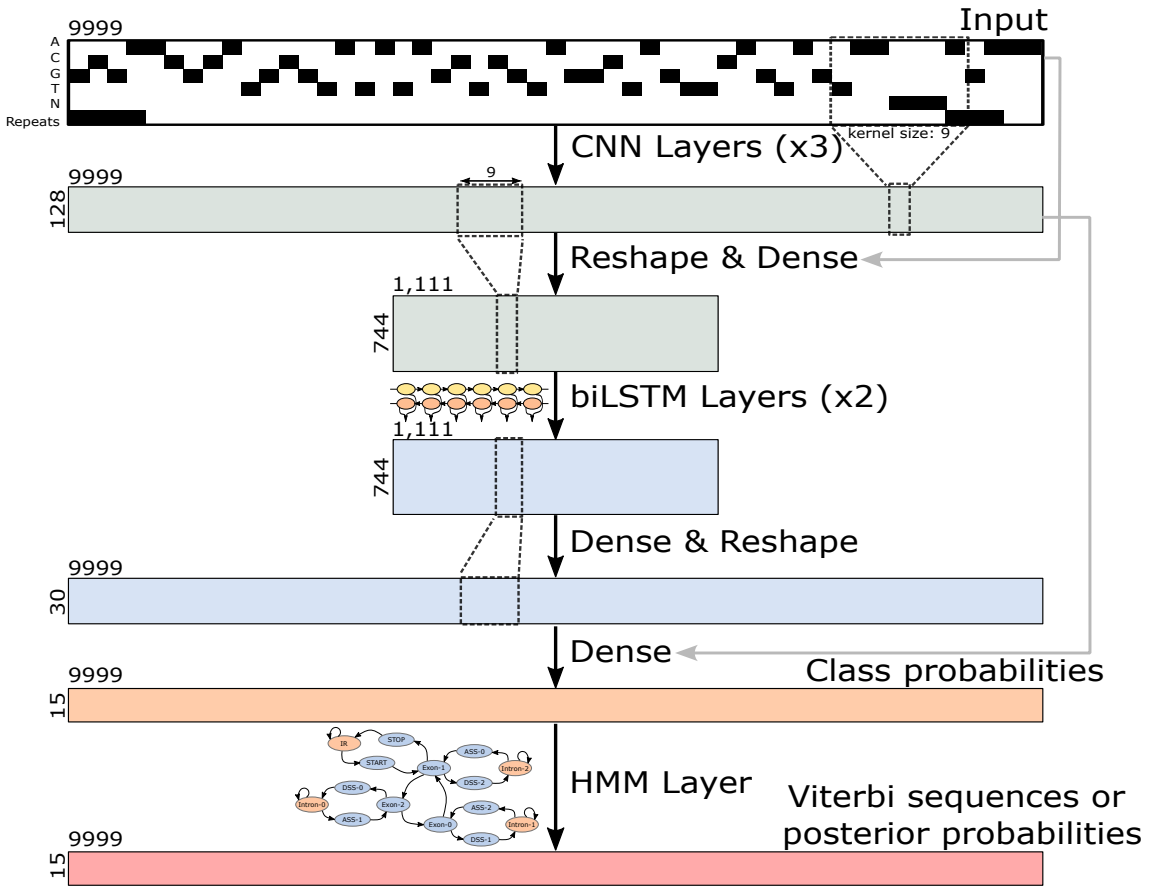
University of Greifswald

Lars Gabriel, Felix Becker, Katharina Hoff, Mario Stanke

Contributing to clade parameters:
Tomás Bruna, Asees Kaur, Anish Krishnan, Felix Ortmann, Asaf Salamov, Igor Grigoriev, Samuel Talbot, Christopher Wheat, Richard Krieg

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 than Helixer
- higher accuracy
- (*ab initio* prediction)
- container for A100 GPU

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

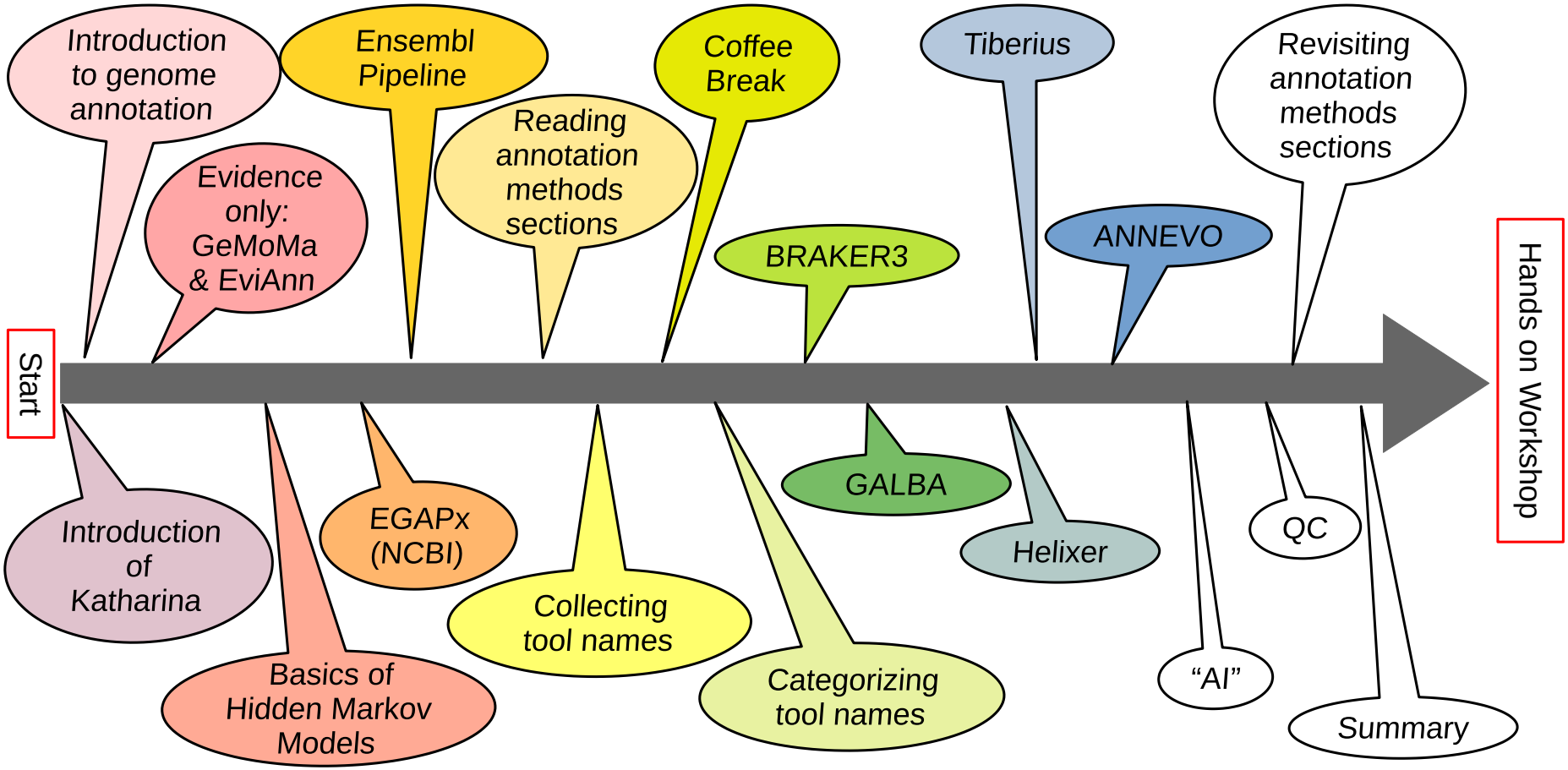
Training Tiberius for clades

Selected examples

Clade	# Train Species	Test species
Mammals	32	*Bos taurus*, *Delphinapterus leucas*, *Homo sapiens*
Eudicots	25	*Arabidopsis thaliana*, *Eschscholzia californica*, *Mimulus guttatus*
Monocots	20	*Brachypodium stacei*, *Freycinetia multiflora*, *Sorghum bicolor*, *Urochloa brizantha*
Insecta	100	*Bombyx mori*, *Colias croceus*, *Danaus plexippus*
Diatoms	10	*Phaeodactylum tricornutum*, *Thalassiosira pseudonana*
Vertebrates	66	*Archocentrus centrarchus*, *Gallus gallus*, *Homo sapiens*, *Zootoca vivipara*

Supervised Training Procedure

- Requires GPU and annotated genomes
- Training time can take up to 15 days
- ... more parameter sets are available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ANNEVO: HiC inspired fast genome annotation

Image: ChatGPT by OpenAI

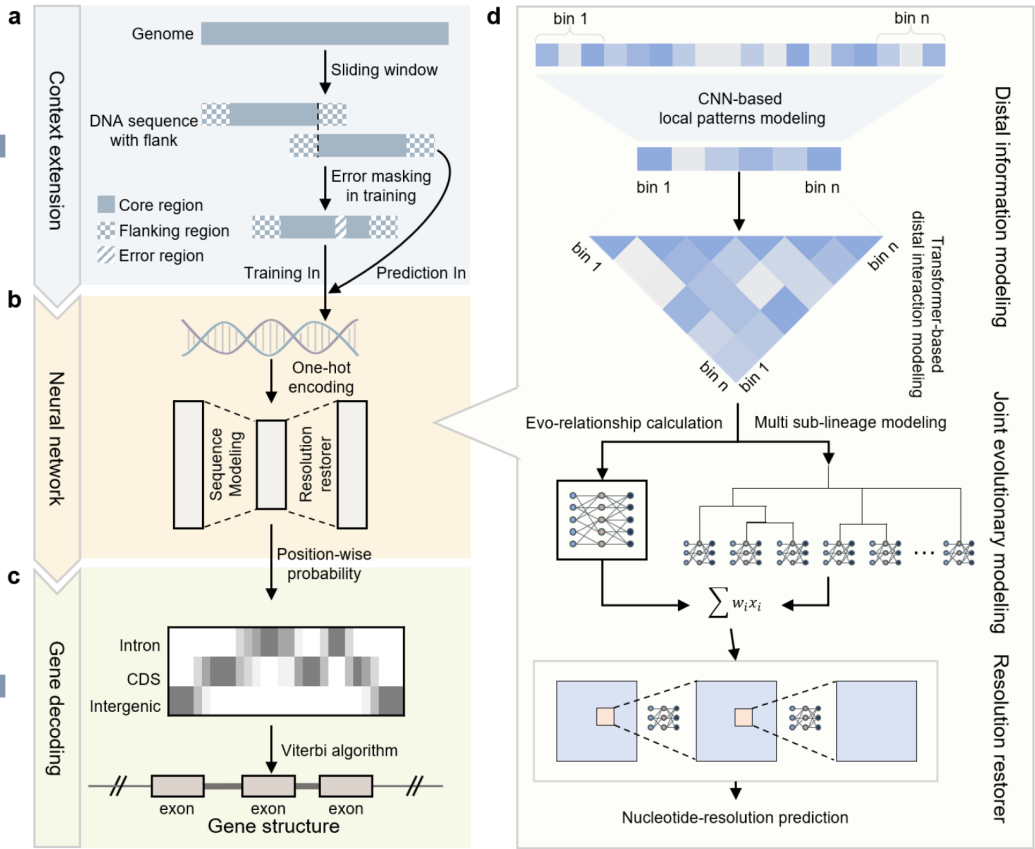
ANNEVO: https://github.com/xjtu-omics/ANNEVO

Highly accurate ab initio gene annotation with ANNEVO

Kai Ye, Pengyu Zhang, Tun Xu, Songbo Wang, Xiaofei Yang, Peisen Sun, Peng Jia, Bo Wang, Stephen Bush, Zemin Ning.

Under review at Nature Methods

Context aware



Hi-C inspired

Evo-aware

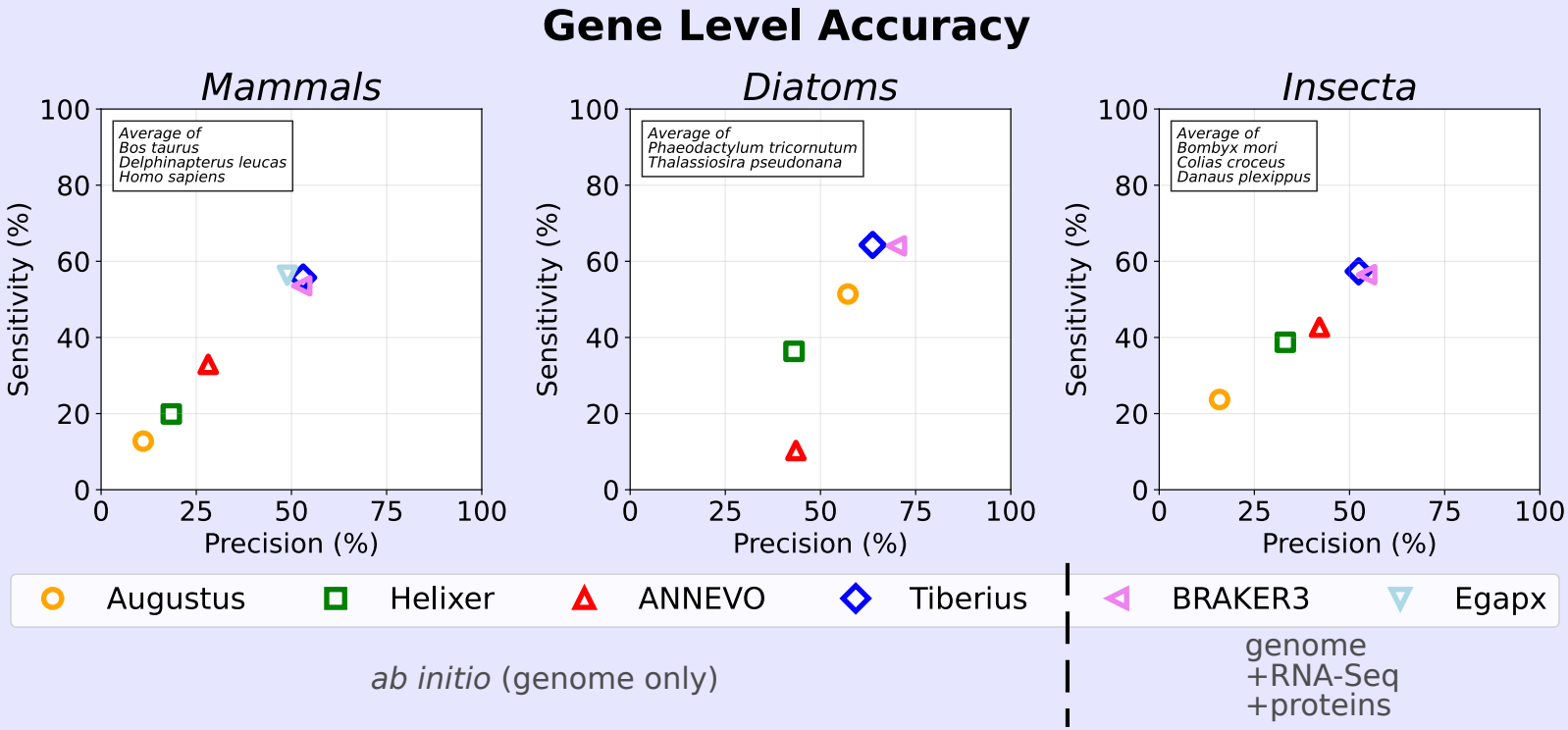
Gene structure constraints

No RNA, No protein, No repeat masking

Image: courtesy of Kai Ye

Benchmark: Deep Learning *ab initio* vs. traditional pipelines with evidence

Average accuracy in Tiberius' test species



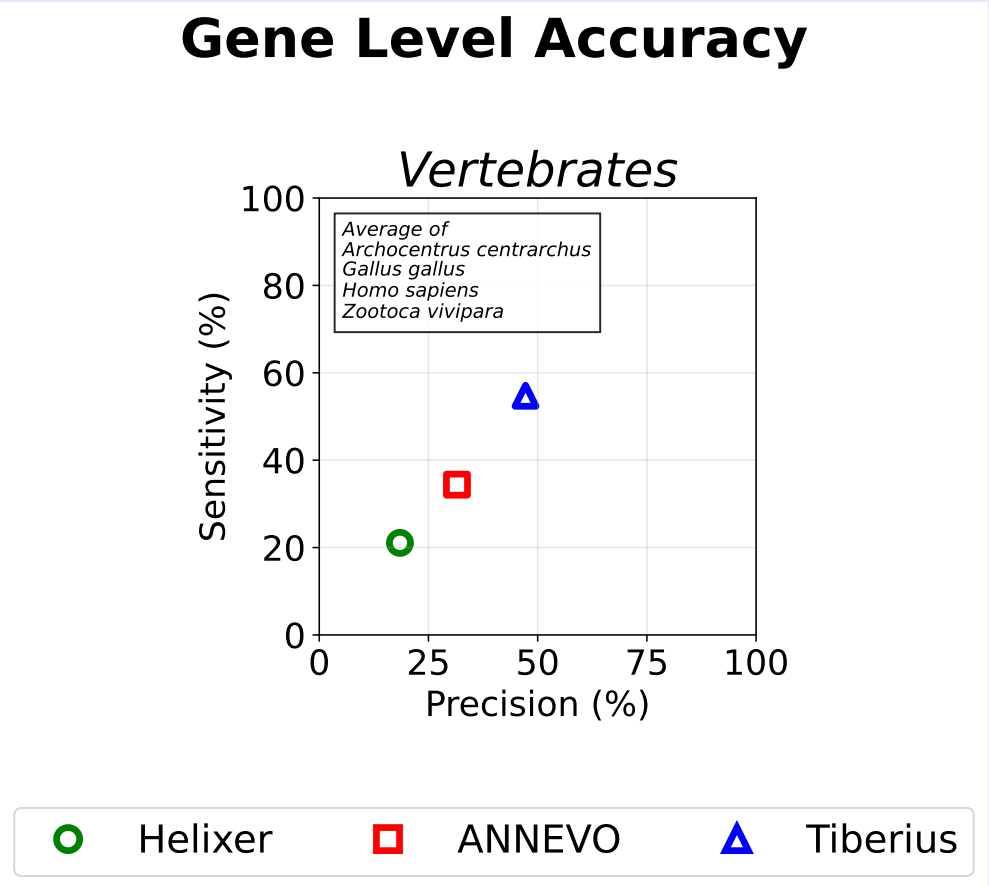
Non-dedicated model used for ANNEVO (invertebrates) & Helixer (landplants) on diatoms

Take home message

- *ab initio* accuracy of Tiberius comparable to BRAKER3 & EGAPx
- much faster, no extrinsic data, only one isoform per gene

Benchmark: *ab initio* gene prediction tools

Average accuracy in Tiberius' test species

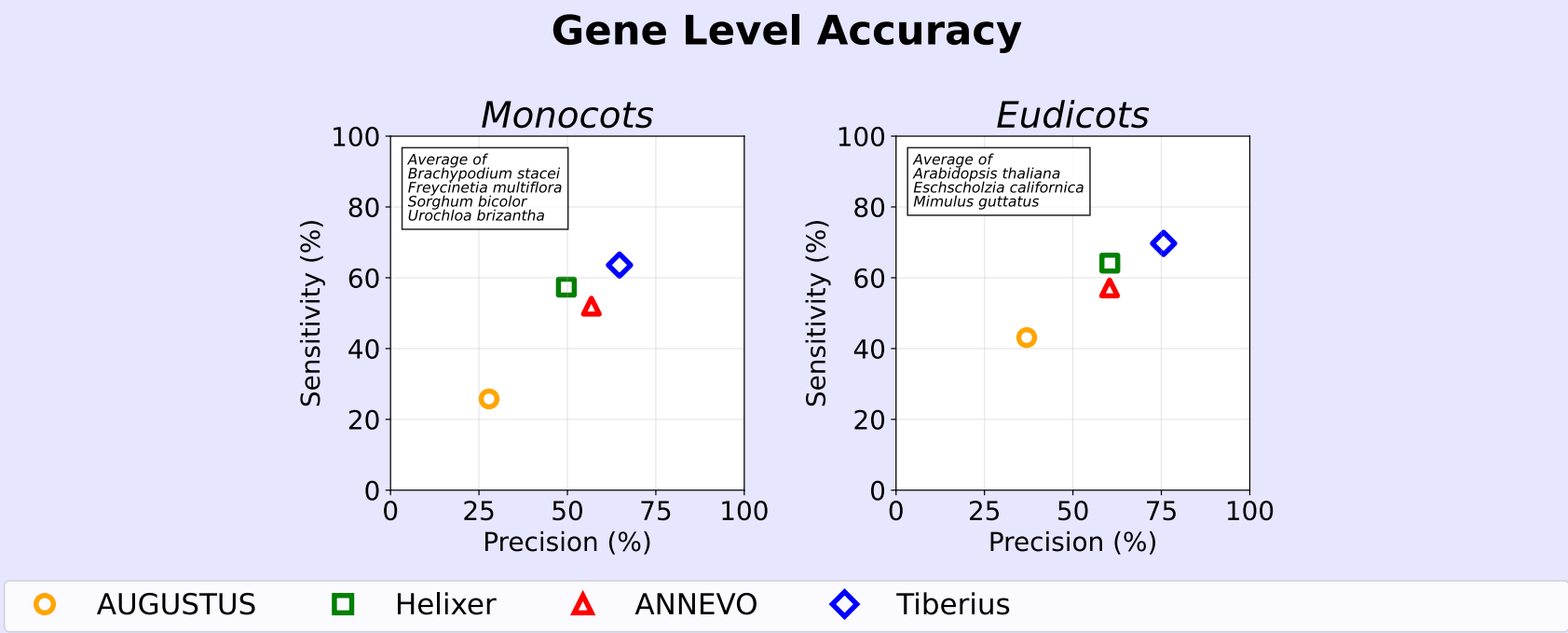


Test species include

- fish
- bird
- mammal
- reptile

Benchmark: *ab initio* gene prediction tools

Average accuracy in Tiberius' test species



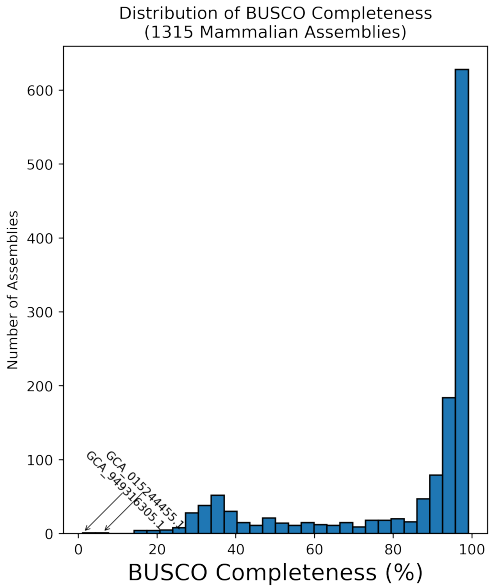
Tiberius enables large scale *ab initio* annotation of genomes

Mammalian Genomes

- 1314 assemblies from 788 species
- 344 primate and 970 assemblies not from primates
- Hiram Clawson (UCSC) has added 701 annotation sets to GenArk browser (`https://hgdownload.soe.ucsc.edu/hubs/`)

Download:

`https://bioinf.uni-greifswald.de/bioinf/tiberius/genes/tib-tbl.html`



Tiberius Gene Predictions on Mammals

Below tables contains the predictions of the gene finder Tiberius on 344 primate assemblies and 970 mammalian assemblies that are not from primates.
Tiberius v1.1.3 (GitHub commit d0c17c5) was run with this command line:

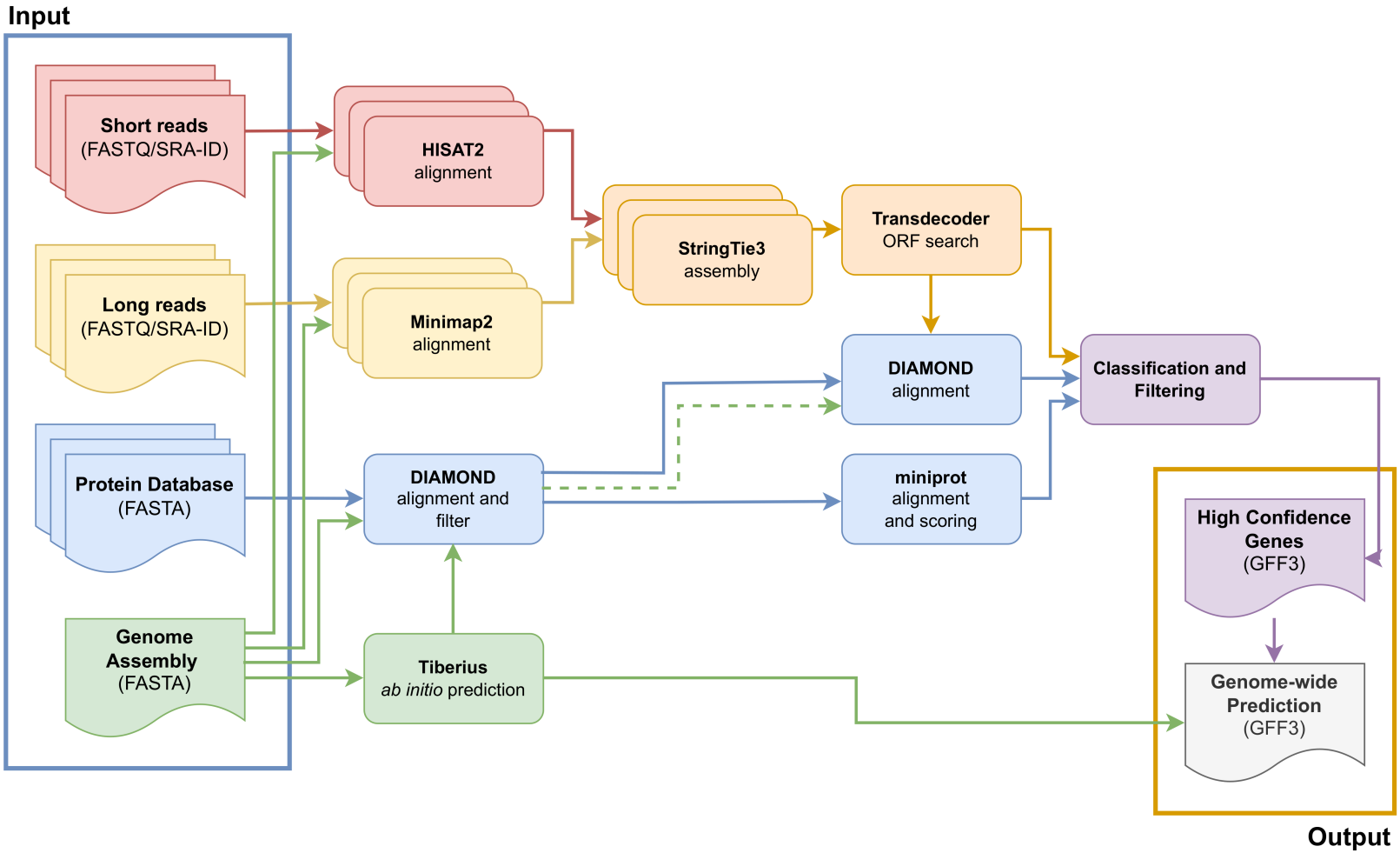
```
python3 tiberius.py --genome ${ACC}.fa --out ${ACC}.gtf
```

where ACC is the accession. The genomes were downloaded from Genbank in December 2024 and January 2025. Mario Stanke, Greifswald, Germany, February 2, 2025

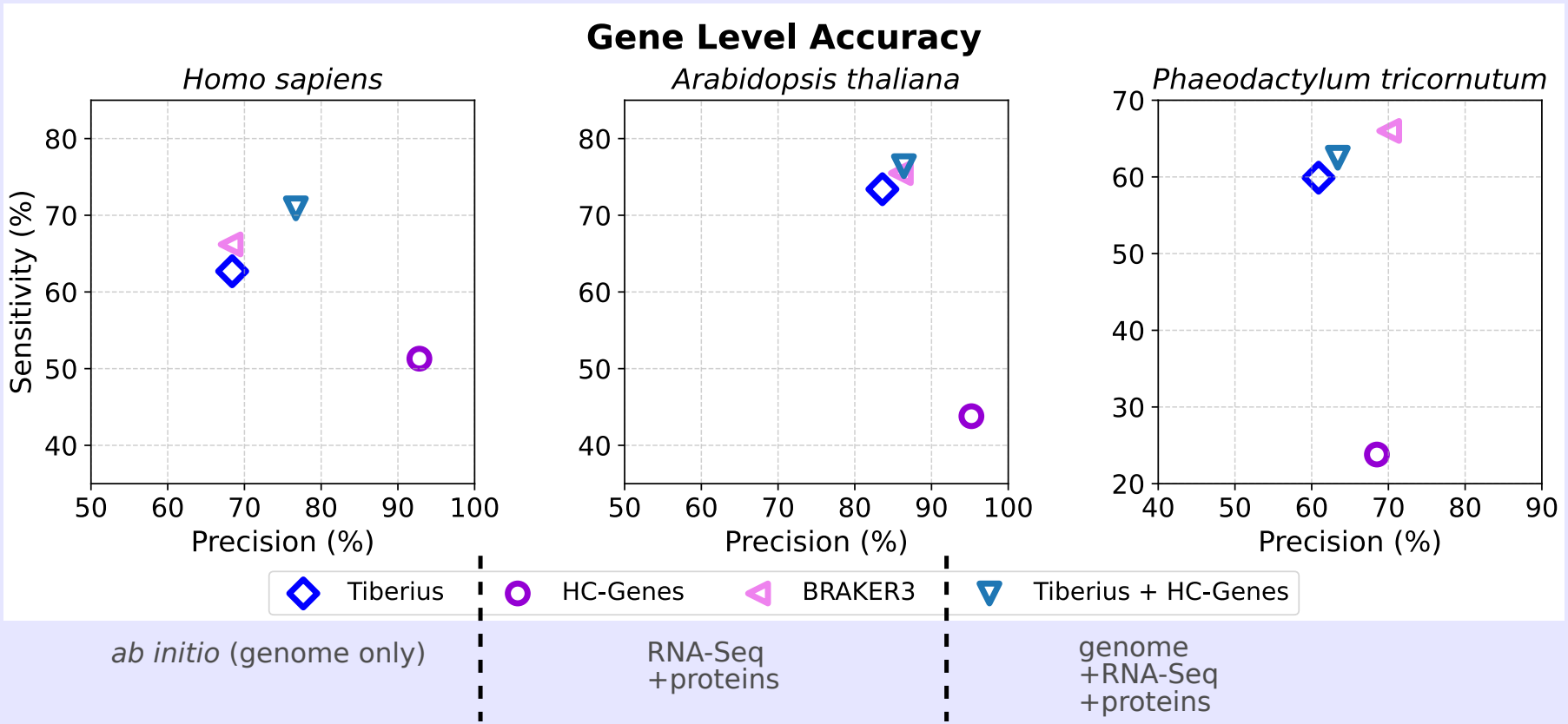
#	Accession	Species	Clade	Tiberius				Reference	Alternate species name	Genome Size
				Number of Genes	GTF	AA	codingseq			
1	GCA_001443585.1	Acinonyx jubatus	mammals	17566	GTF	AA	codingseq		cheetah	2372536860
2	GCF_027475565.1	Acinonyx jubatus	mammals	19294	GTF	AA	codingseq	reference genome	cheetah	2377417351
3	GCA_004027535.1	Acomys cahirinus	mammals	14398	GTF	AA	codingseq		Egyptian spiny mouse	2306070819

Image: Gemini with NanoBanana

Tiberius evidence processing pipeline

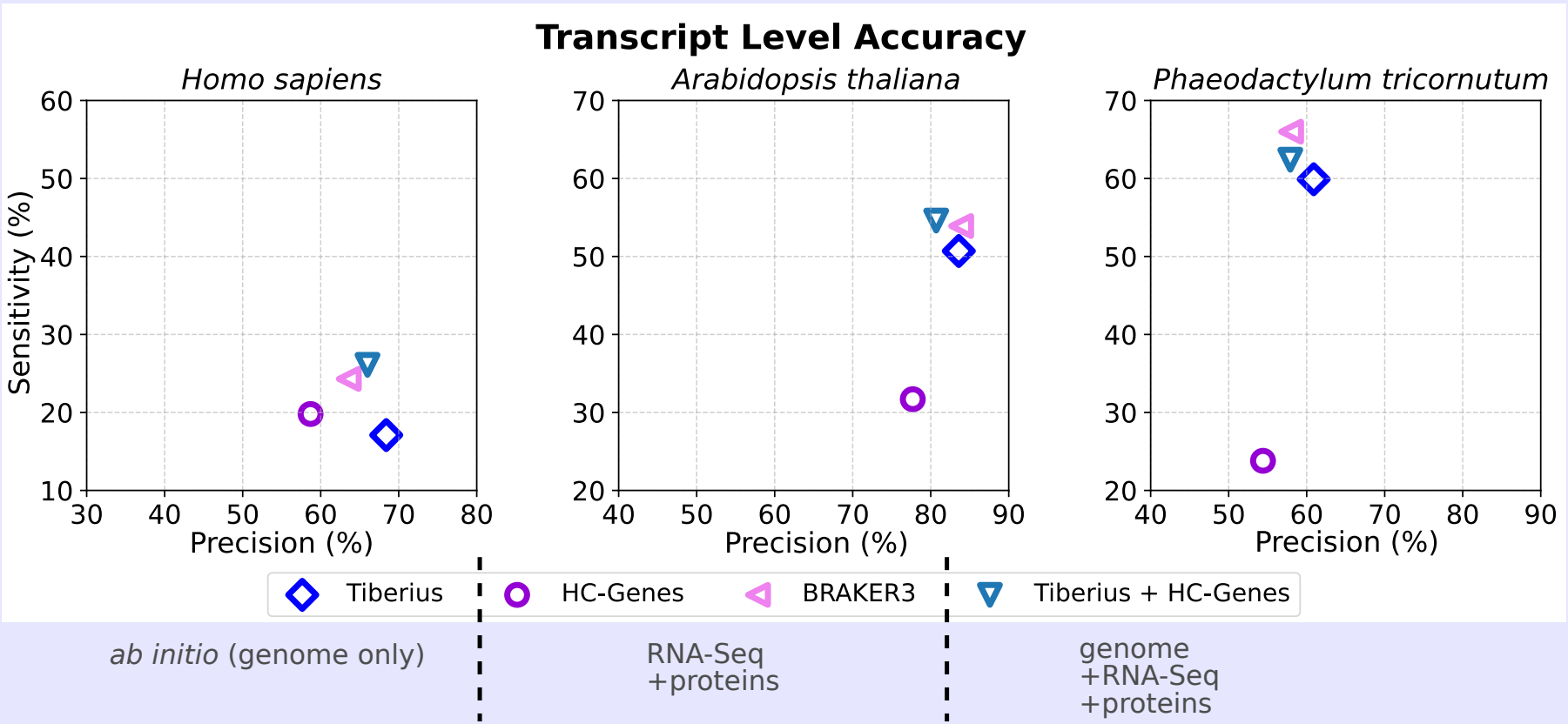


Benchmark: Tiberius with extrinsic evidence



Proteins: OrthoDB excluding proteins from species of the same order.

Benchmark: Tiberius with extrinsic evidence



Proteins: OrthoDB excluding proteins from species of the same order.

Runtime comparison

Runtime (min:sec)

Species	GPU ANNEVO	GPU Tiberius*	GPU Helixer	CPU BRAKER3	CPU HC-Genes*
Arabidopsis thaliana	3:49	6:45	13:12	242:02	202:28
Homo sapiens	87:09	116:02	567:36	2663:15	233:43
Phaeodactylum tricornutum	0:46	3:29	8:24	307:20	209:40

Conditions

- Runtime for whole-genome annotation
- GPU-based methods: 1x A100
- CPU-based methods: 100 threads

* Can be further parallelized with Nextflow on an HPC

Take home message

- using evidence increases accuracy of Tiberius
- runtime shorter than BRAKER3 but substantial
- very new, we can't cover it in the hands-on session this year

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

Tiberius *ab initio*

```
tiberius.py --genome genome.fa --out tiberius.gtf \
        --model_cfg eudicots.cfg
```

Tiberius with evidence

```
tiberius.py --params_yaml parameter.yaml \
        --nf_config nextflow.config
```

Tiberius via singularity

```
singularity build tiberius.sif \
        docker://larsgabriel23/tiberius:latest
singularity run --nv tiberius.sif \
        tiberius.py --genome genome.fa \
        --out tiberius.gtf  \
        --model_cfg eudicots.cfg
```

Tiberius is under MIT License

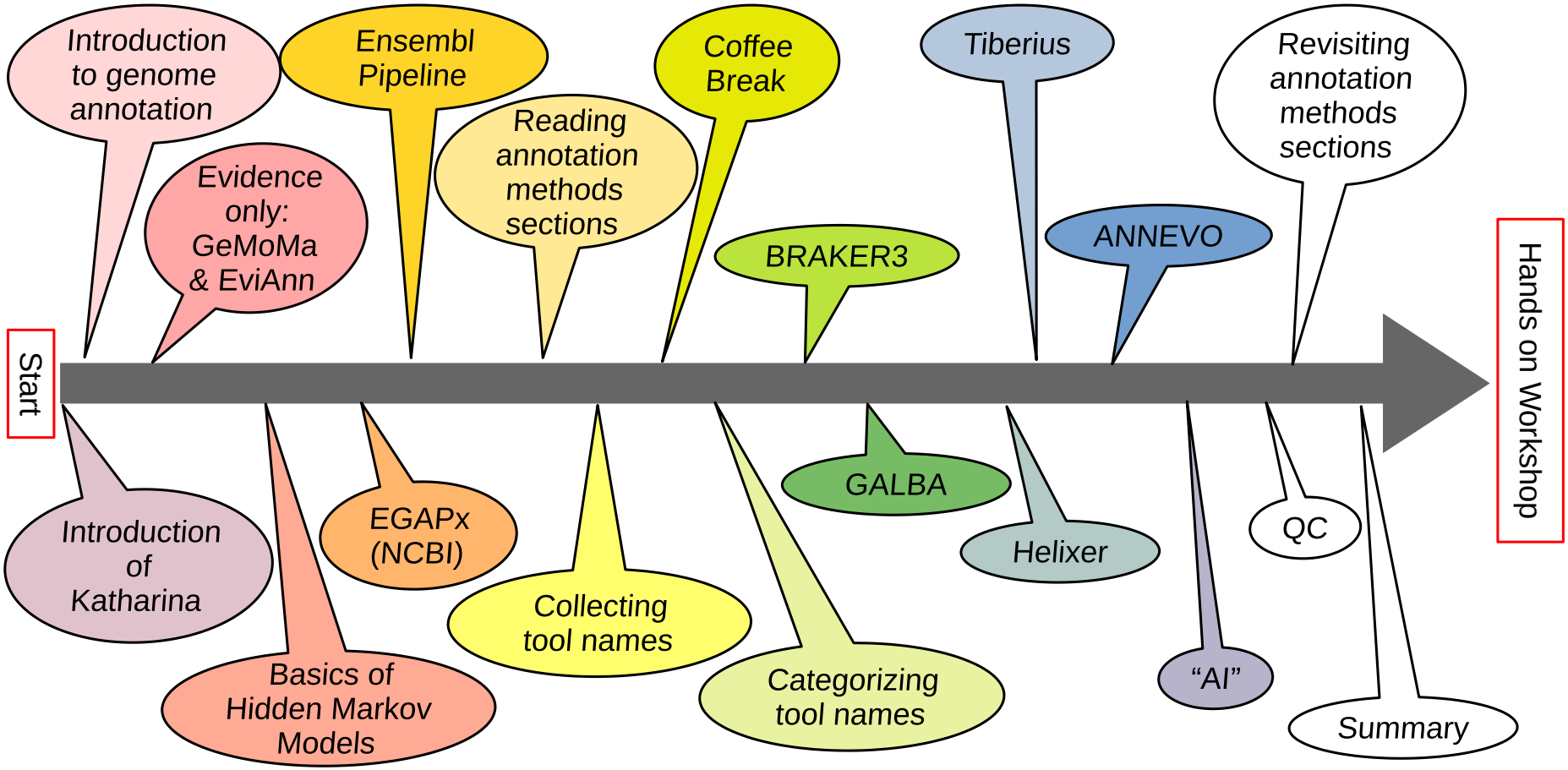

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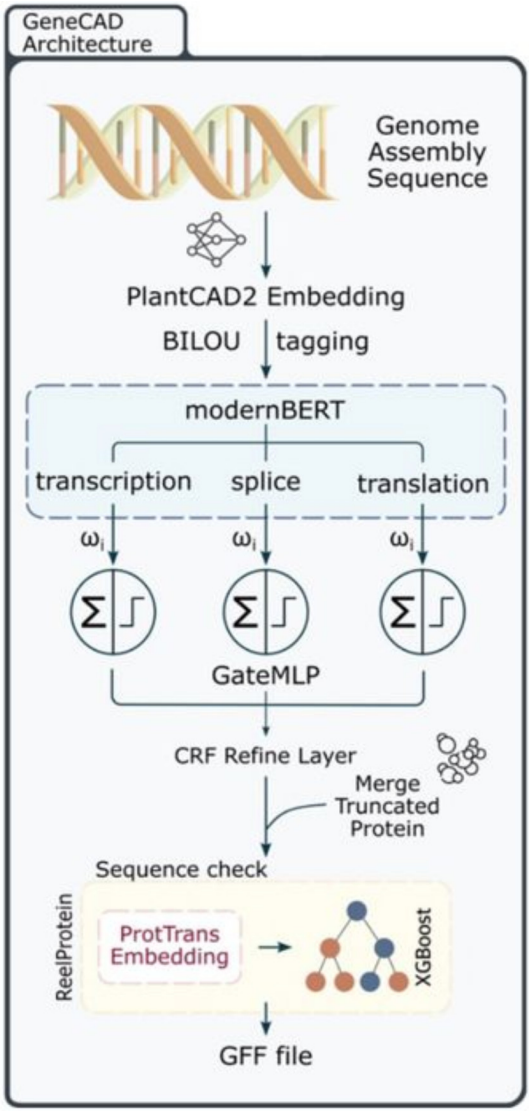
Image: Gemini with NanoBanana

GeneCAD: Plant Genome Annotation with a DNA Foundation Model

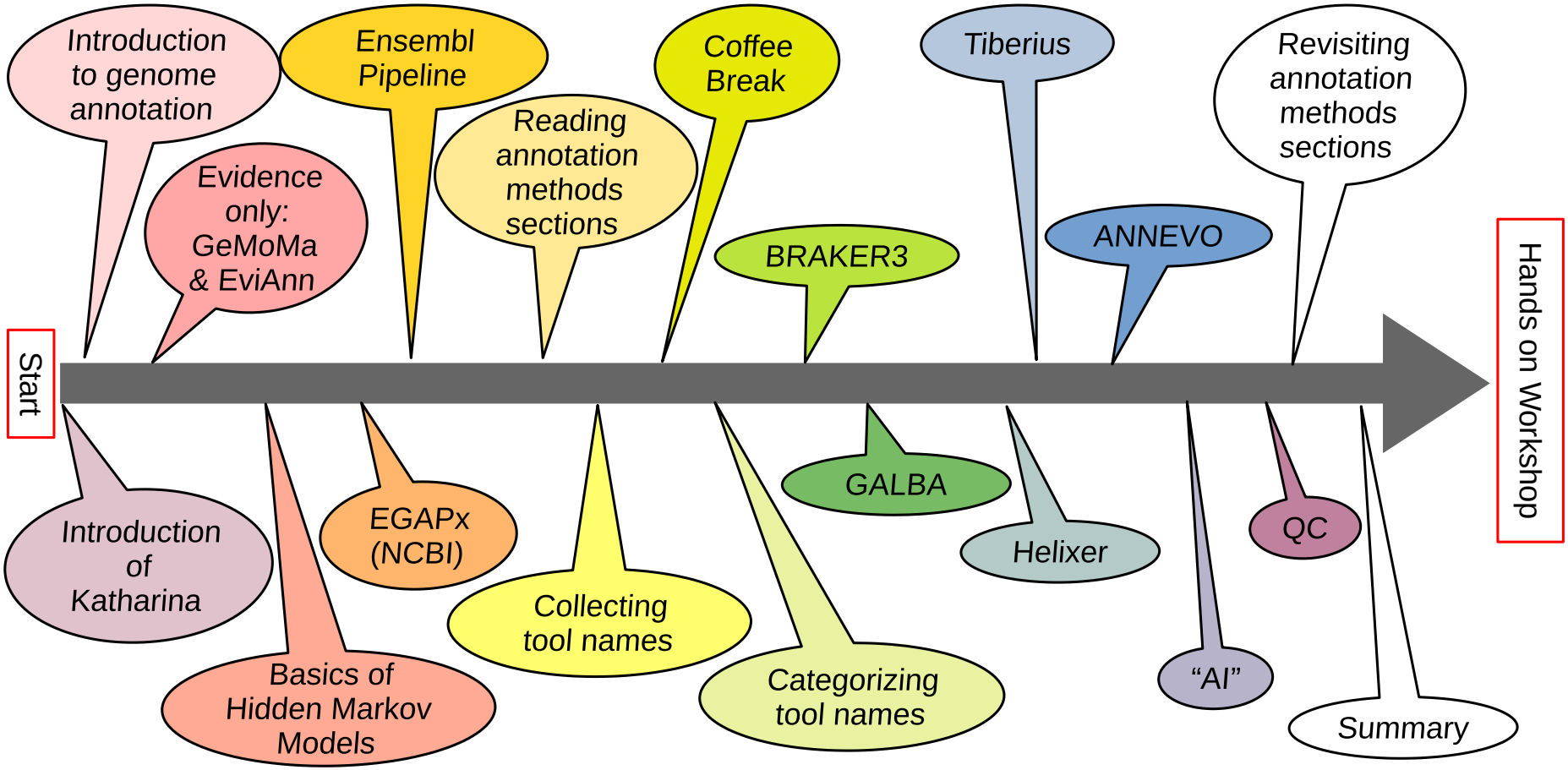
Zong-Yan Liu, Ana Berthel, Eric Czech, Michelle Stitzer, Sheng-Kai Hsu, Matt Pennell, Edward S. Buckler, Jingjing Zhai

doi: https://doi.org/10.1101/2025.10.31.685877



- for plants only
- CDS locus accuracy unclear
- demonstrates potential

Image: Liu *et al.* (2025), Fig. 1b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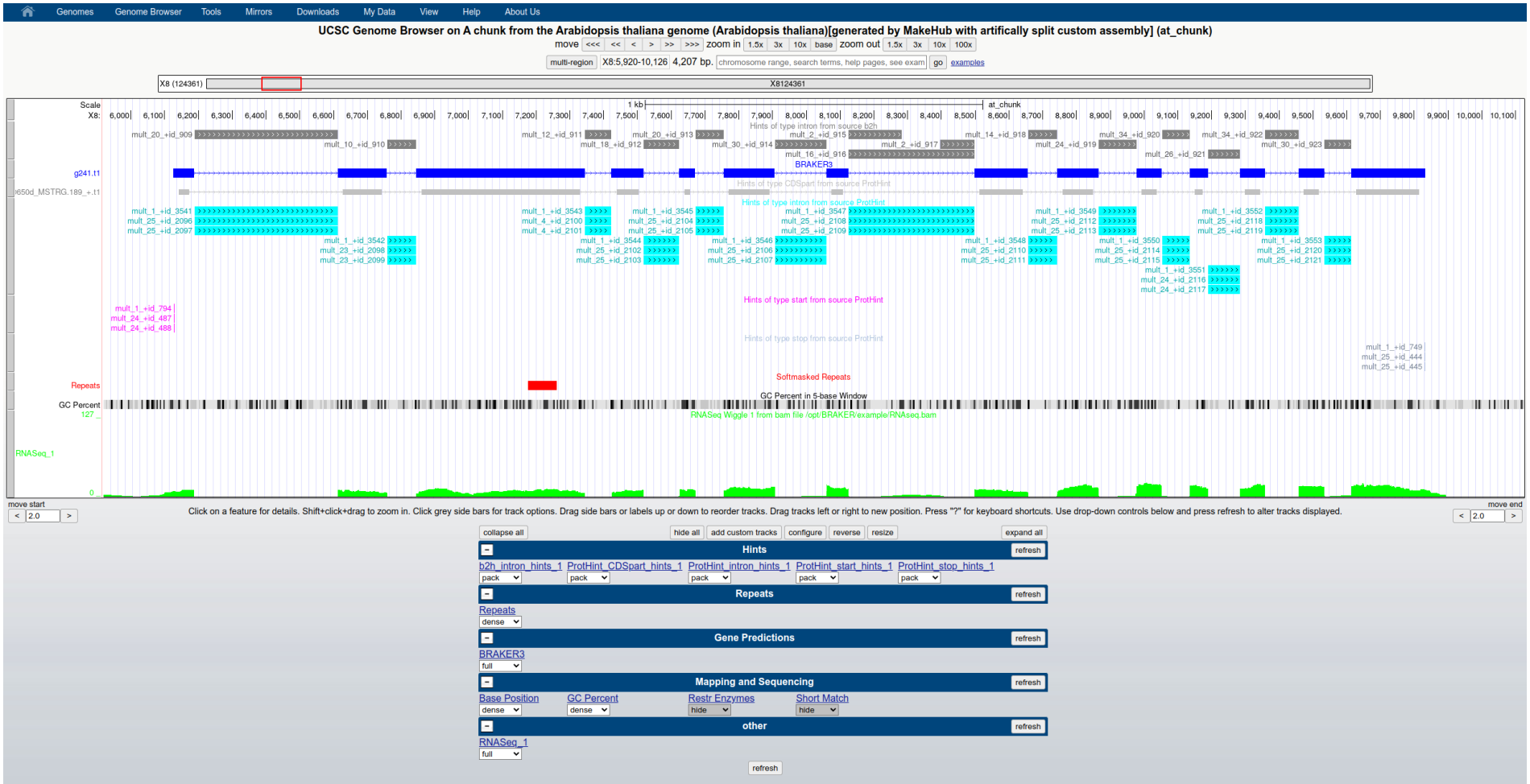
Did we do a good job?

Genome Browsers

Visualize your annotation in context with evidence

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

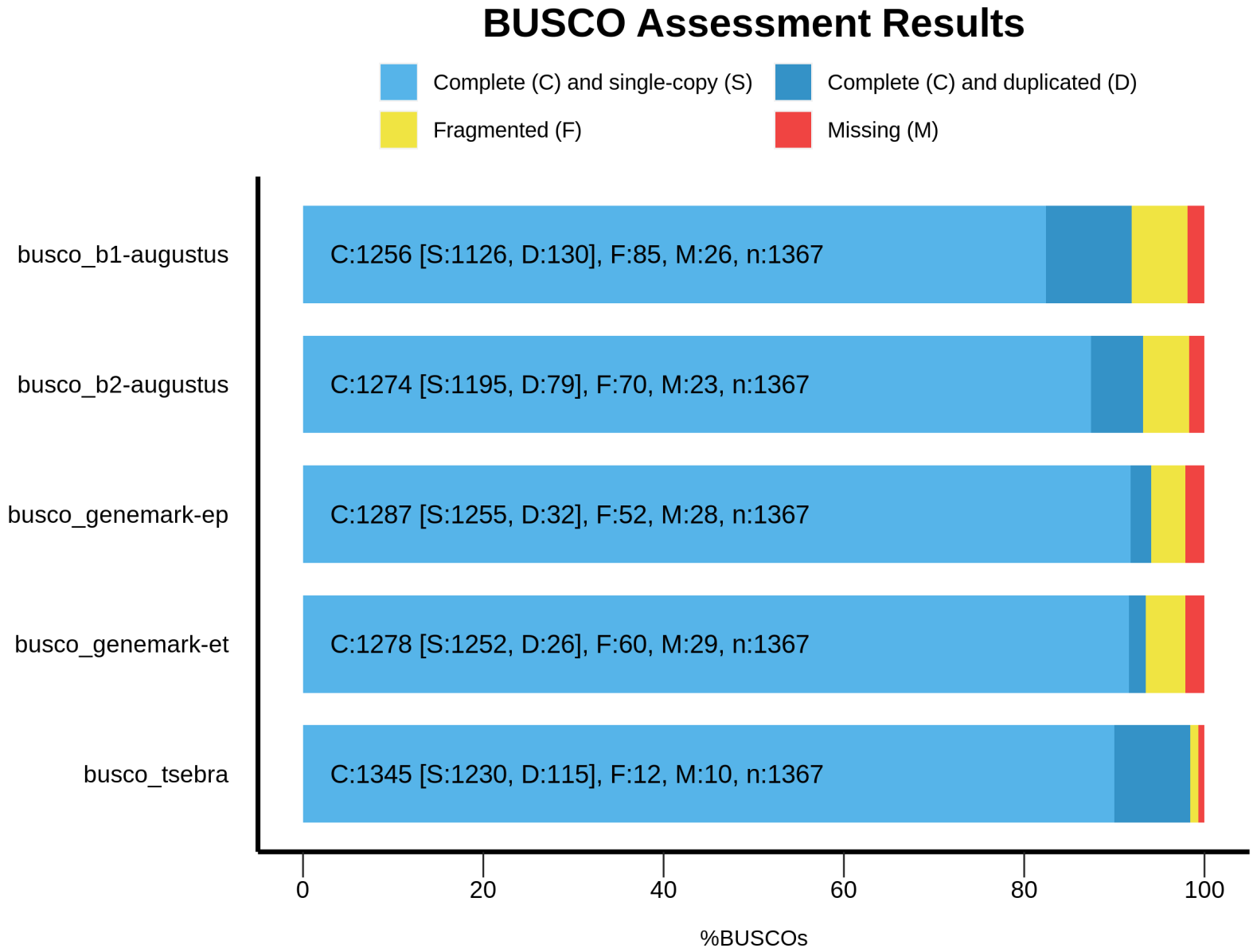


Describe your annotation

- 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
- ...

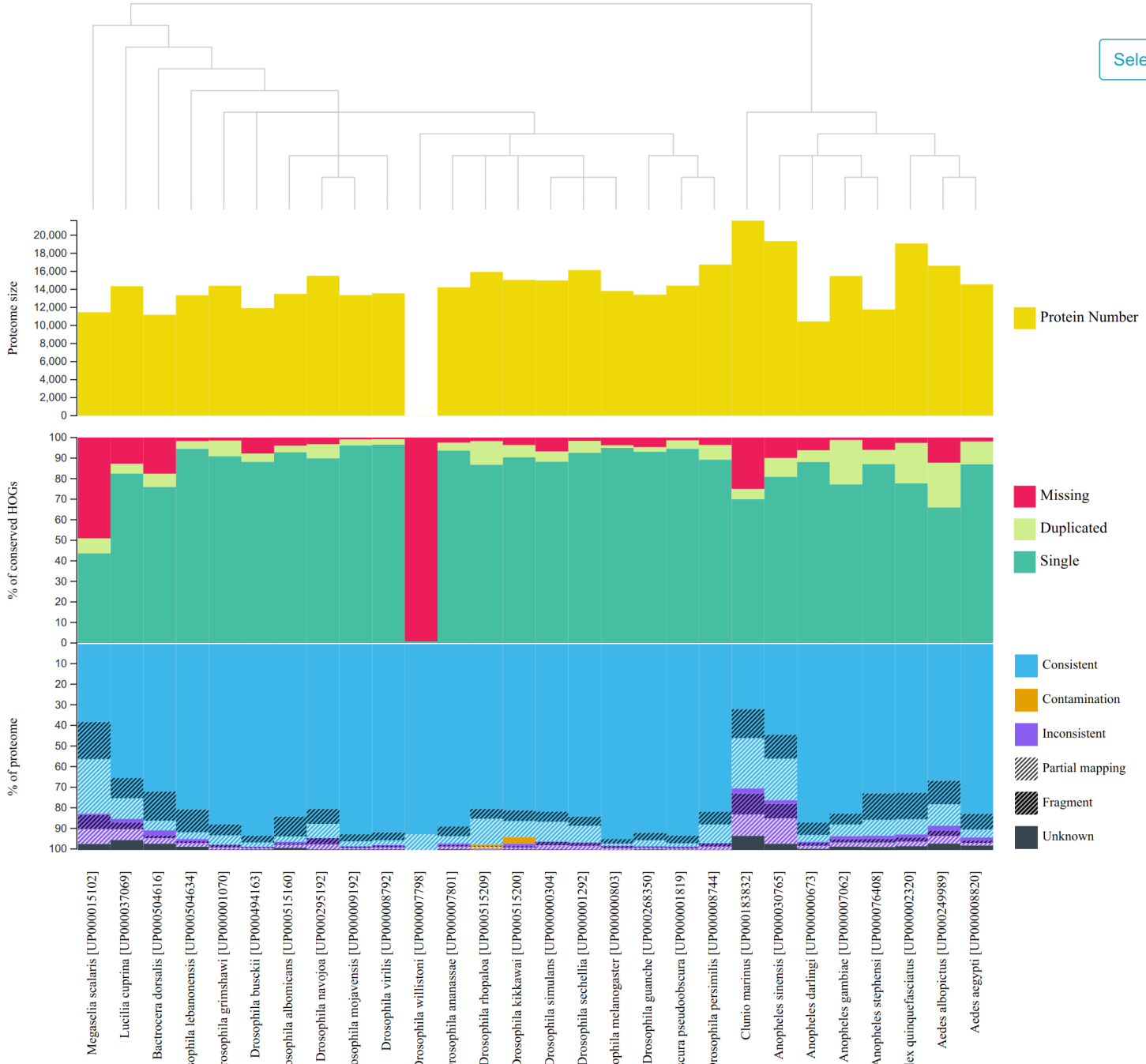
If possible, compare to annotated close relatives.
Consider effect of individual annotation pipelines.

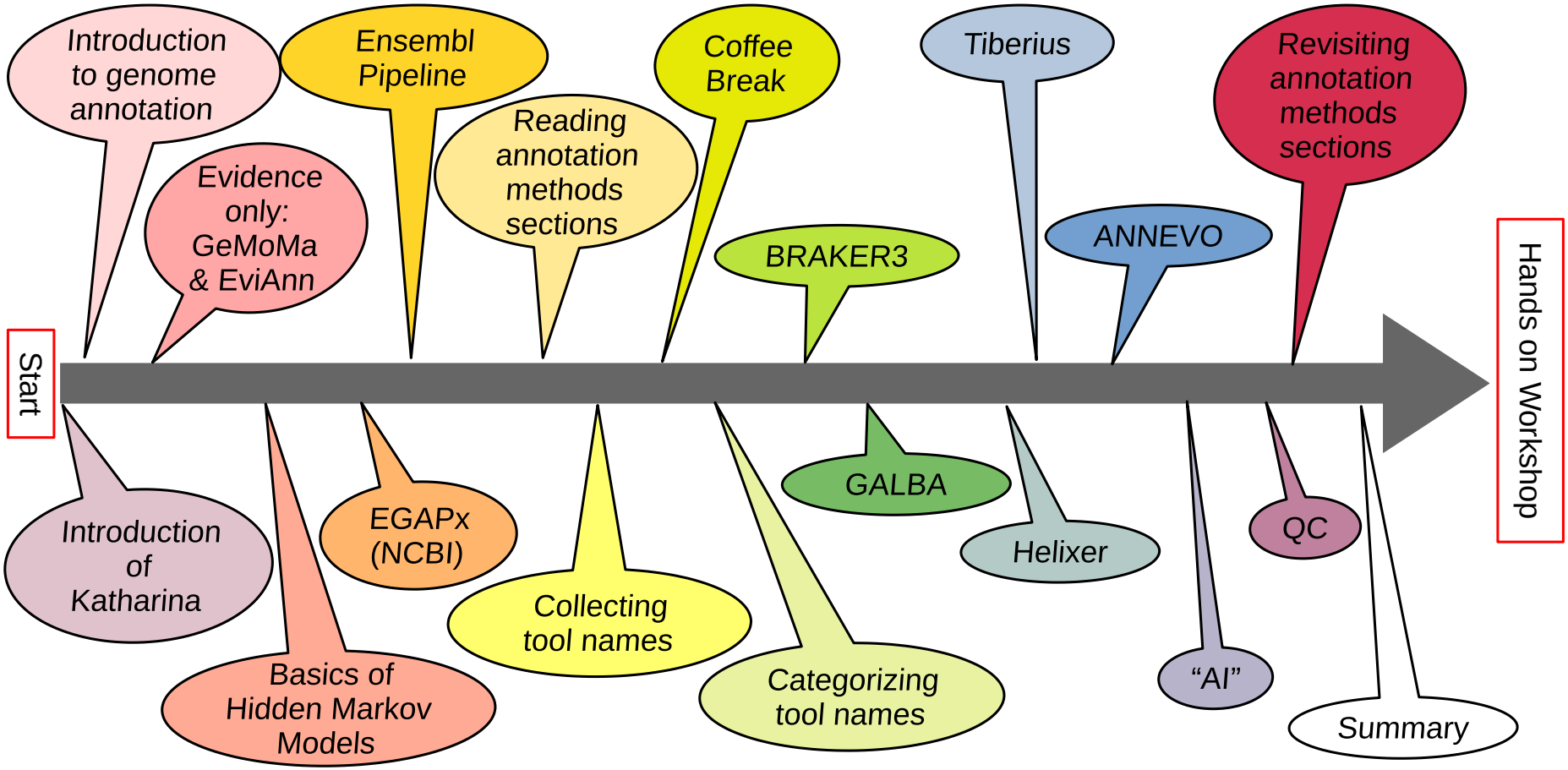
BUSCO: Sensitivity in clade-specific conserved genes



Beware! BUSCO completeness does not warrant correct gene structures!

OMArk: sensitivity, contaminations, & more

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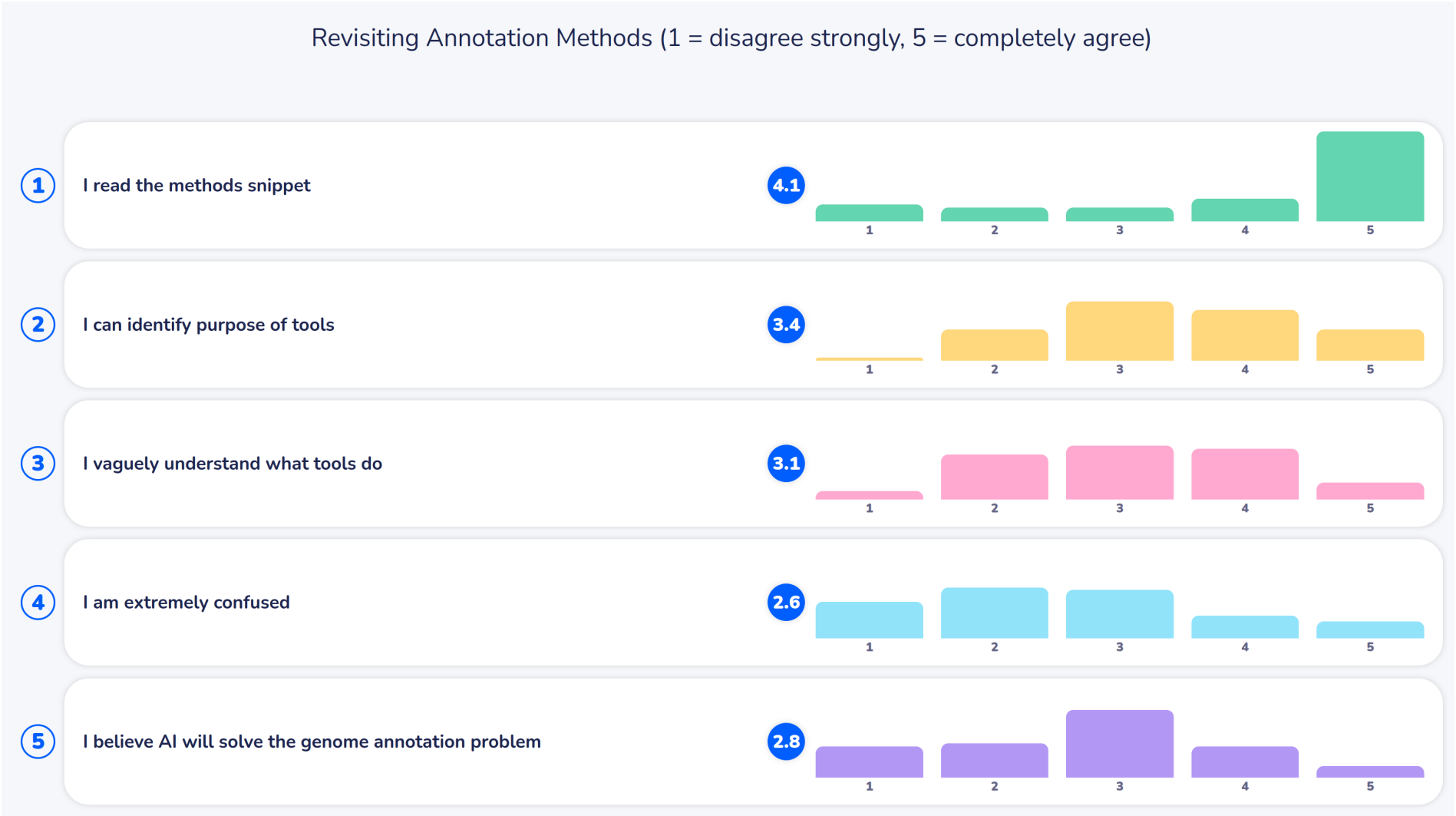
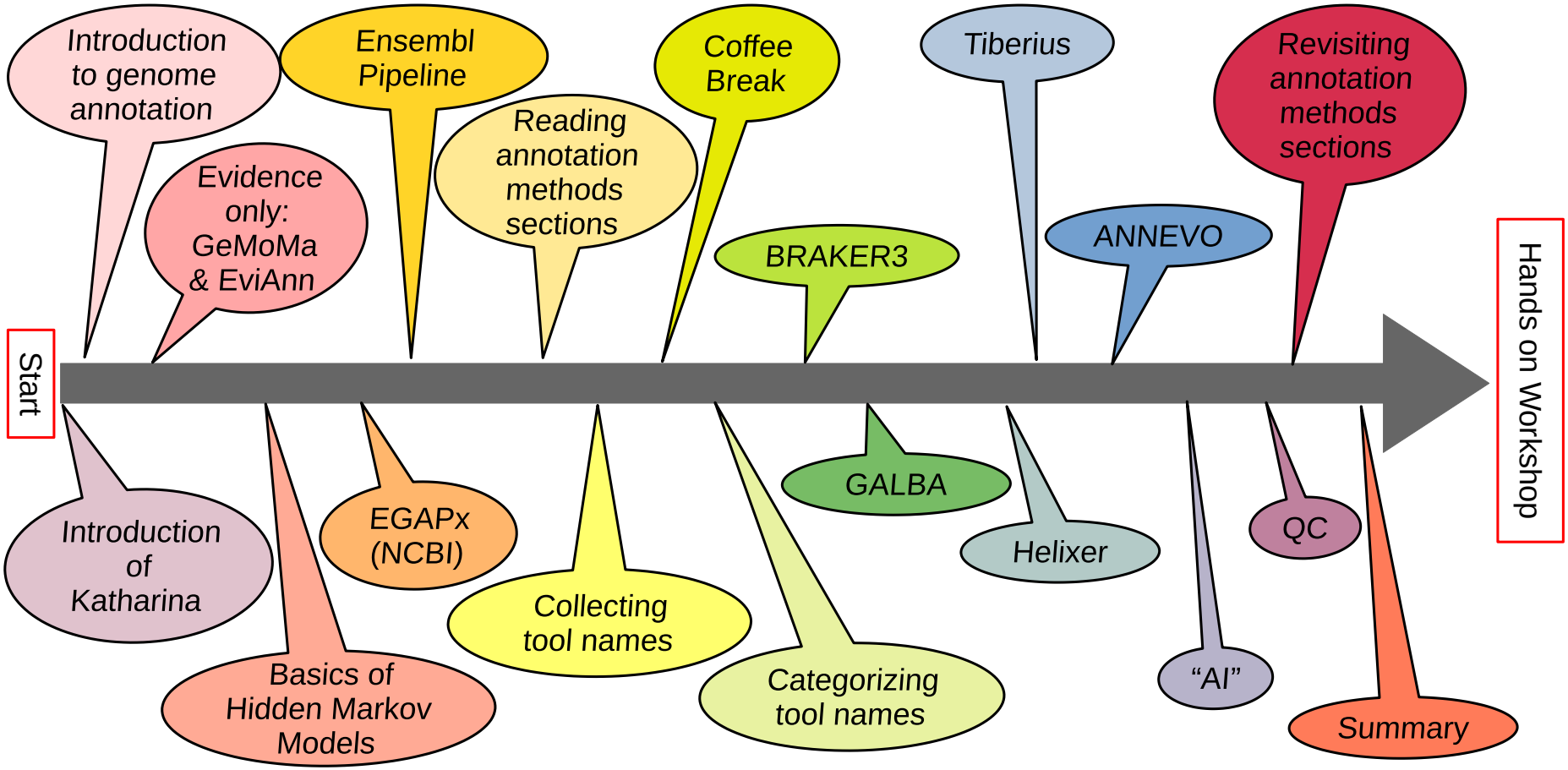
Revisiting annotation methods sections

Your tasks

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

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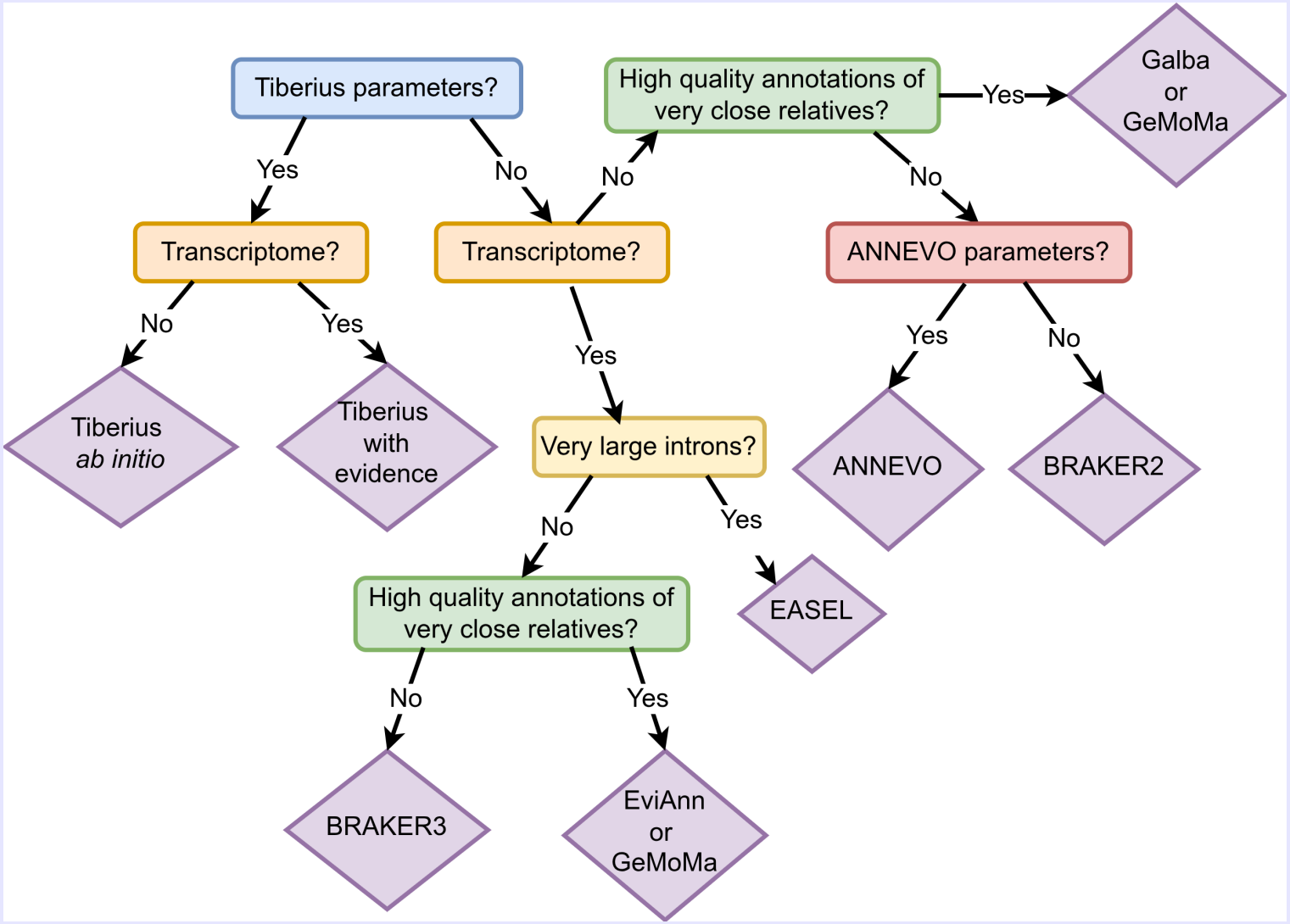
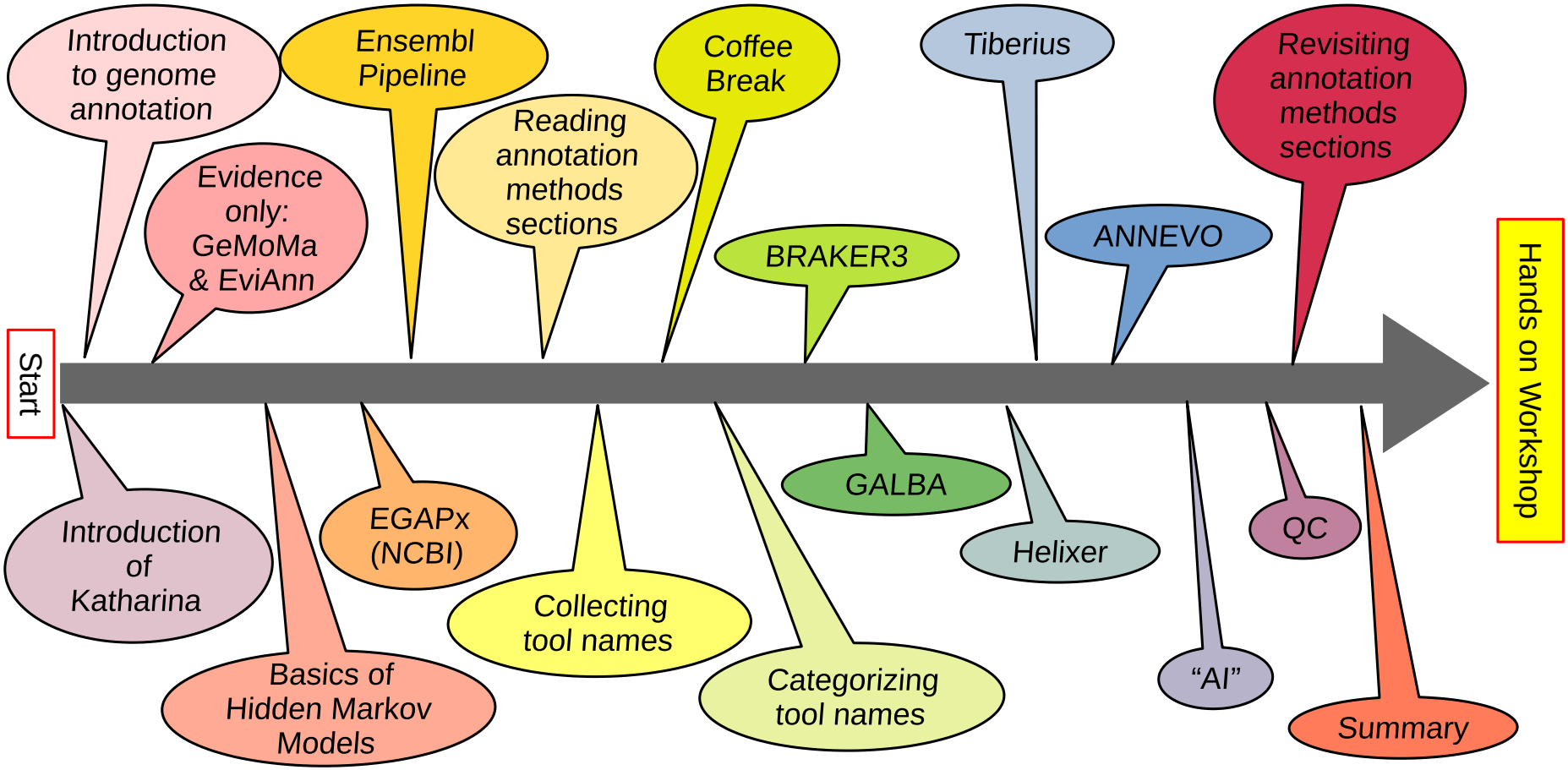
Most important stuff on genome annotation

- structural genome annotation in eukaryotes is hard
- Hidden Markov Models (& CRFs) remain essential
- evidence improves accuracy
- popular community annotation pipelines:
  1. BRAKER
  2. GALBA
  3. EGAPx
- deep learning is changing the field
  1. Tiberius most accurate
  2. Tiberius can become even more accurate with evidence
- "looking nice" is not always "correct"
- BUSCO & OMArk
- high marker gene detection rate $\neq$ high accuracy

Navigating the maize of tools

What would Katharina try?

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