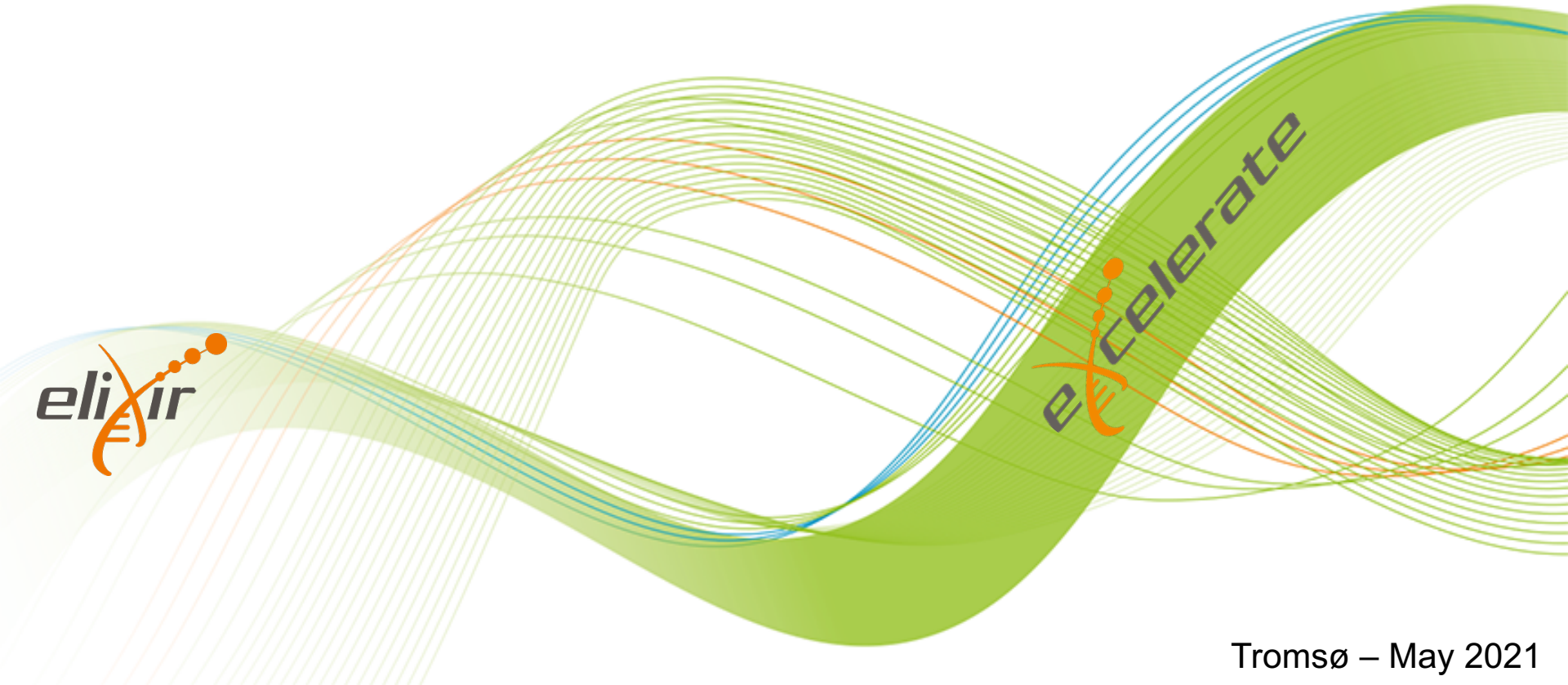
Lucile Soler, PhD
Jacques Dainat, PhD

# Methods in genome annotation




Tromsø – May 2021

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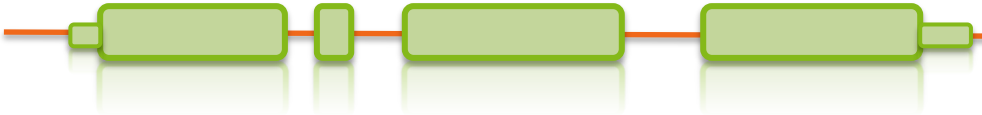
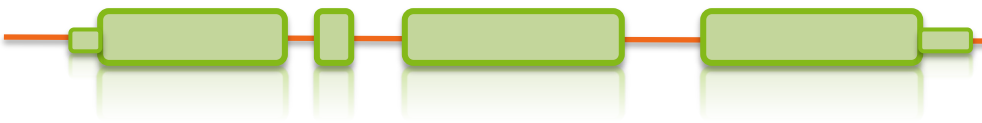
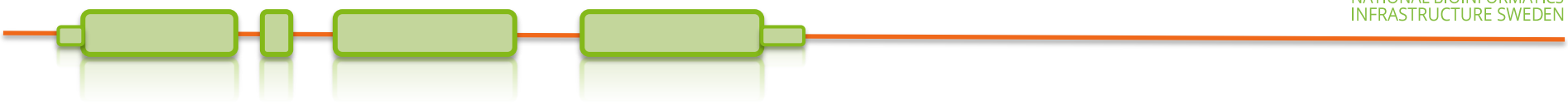




NATIONAL BIOINFORMATICS INFRASTRUCTURE SWEDEN

# 1. Introduction



# What is annotation?

Structural annotation:

Find out where the regions of interest (usually genes) are in the sequence data and what they look like.
=> **Gene prediction** / **Gene Finding**

+

functional annotation:

Find out what the regions do. What do they code for?

*It is the **annotation** that bridges the gap from the sequence to the biology of the organism*

# Introduction: Formats

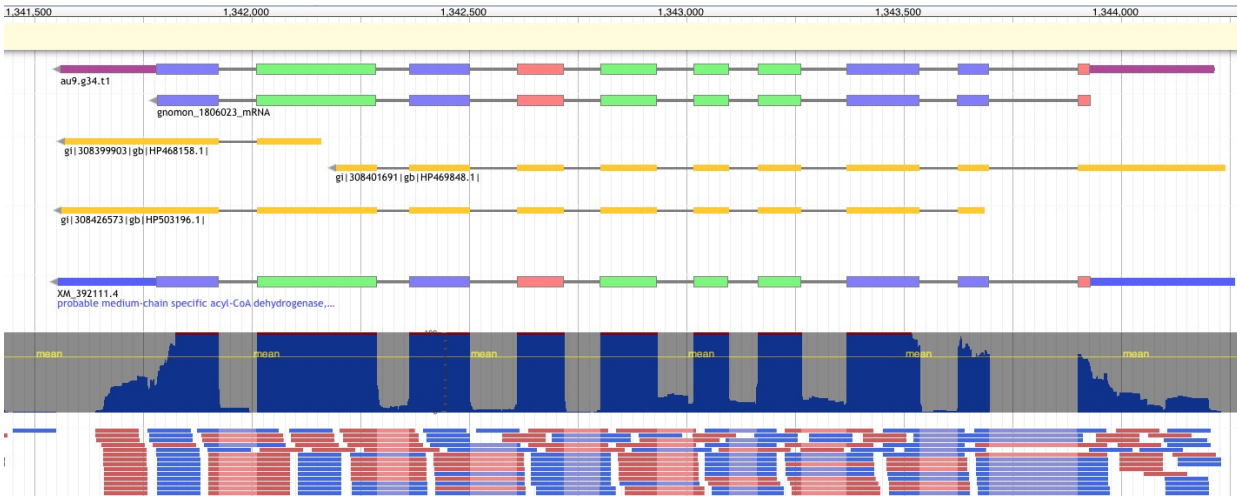
From a genome…

**FASTA**

```
>scaffold_26
AGTCACACACCCTTCAGCTTACACCCTGACTGCAGCCCTTACTCAAAACA
TTCCAGCCAGGAAGATGCTCCGACACAGCTTCTGGATGCCGCTCCTCGAC
GTCGAACGGCCCGCGCCGGGAAAATCGGCAGCGTCGGTGACCGCGGAGAT
CCGAAGCCGCCTCGGGGACCTGCGAGACAACGGGAGGCGGTCAACGAGAC
GCCGAGGGCTGGGAGTTATTCCCACACCGGGCCCGTAAGTTTTCTACCCA
AAAACCCATAGAAAAGAGATGAACCACTAAGTTTGATAACTCTTCTACTT
AACCGTGACCCTACGTGCCGGGGCAGGGCAGCTCTGACCCTAAGCGGCAC
ACGAACAAGGTGGTGCGCCCAATATAAACAAAGATGATGCAAGGGCTTGA
AATAAATCTCCGGAAGATTAATTCTCGAGCCCGACACGCTTTGAGGCAGC
GGAACCTACAGAACCACCGCAGTCACGTGAGAAGAGTCTAATACTCTCCA
AAGAGAAGTCCAAGGGAATGGAACGTGAAAAGAAGGTGCTTATCAAAAGC
GAGAAGGAAGATGGATGAGAACATCTTGTGTACTTCTTCTGGTCTCAAAA
AGCAAAAATGTAAAGATGCCAGACTAAGCCCGATCTGAGAAAGTACGCGA
GCAGAGACCCCCGCTGCCGATGTGGCCCAGAACGATGCCGATAAAGCACC
GAGACATAACAAAGCCCTGTGACACACAAGACGATGGACACAAACTACAT
AACACAGACACAAACTAAATGACACAGAGAGAAGTTGAAACTTCTGGGGA
AGTAAACATTTCTGAAACATCTACCAACAATCCGTCCATATATATTTCCA
TTCCACGGGACTCTTGGTTTGATATATGCGTGTTAACAGTAATCCCCGCT
GTAGCAATCACCACTATGCATAATTCATTAATTCTTTGGAGTTGCTGAGT
ATCATCTTATCAGTCTTATTTTTTTCCTTGGCTCTGGTTTCGGGCTTTTT
TTTTTTCTTCTGATAAGATTTTCCAGGAATGTGAAGACCCCCTGCATCCT
TCCCAAACTGACCACCCAAACTACAGACATTCTATAGCATTACATTACAC
AACCTAGGCAAAGTTTTTCTAACATTAAGGAACATGAAAAAAGCCAACAT
CACAATATATTCATAACAATTATGGAACATGCGAAAAGCCAATACCACAG
TACATTTATAACAATACCTCCCTTTTCCTTTCTTTAGAGATCATATGGCT
TGACCGCCGCCTCCTCGCCCGCCACCGCTGAGTACTGCCGTGCCGGAGTC
ACGGAGCCAGTCCCCCGCGGCCCCACCGCCTCCTCGCCCGCCGCCACGGA
GATCGGCTGCGCCACTCCCGAGCTCGGCCGTGCCATCGCCGCCCCCGCCG
GGGTCCCCCGGCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
```

…to an annotated gene

**GTF/GFF**




- 9 columns
- 1 feature = 1 line

| 1. sequence id | 2. source | 3. feature type | 4. start | 5. end | 6. score | 7. strand | 8. phase | 9. attribute(s) tag=value |
|---|---|---|---|---|---|---|---|---|
| Ctg123 | maker | Gene | 1000 | 9000 | . | + | . | ID=gene1; Name=EDEN |

# Introduction: Overview

## The main steps in genome annotation

1 2 3 4 5

QC assembly → Structural annotation → Manual curation → Functional annotation → Downstream analysis / Submission

BUSCO

MAKER Annotate this!

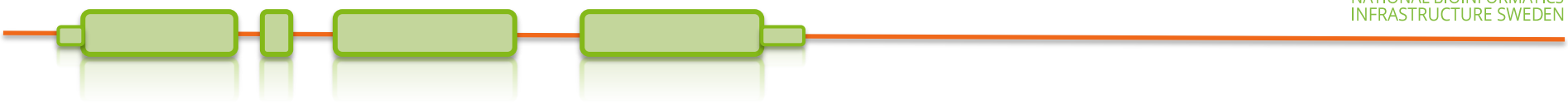
EuGene-EP

Web Apollo

InterPro

GFF3 + FASTA → EMBLmyGFF3 → EMBL format

# Introduction: Structural annotation



## Experimental (ESTs, cDNAs, RNA-seq)

Isolate and clone cognate transcripts (as cDNA), sequence them and compare cDNA with genomic DNA

=> It's the ONLY secure method but:

- Cloning is time consuming.
- Lowly expressed genes are difficult to detect.
- …

## Predictive

- Intrinsic / *ab initio*
- Extrinsic
- Hybrid

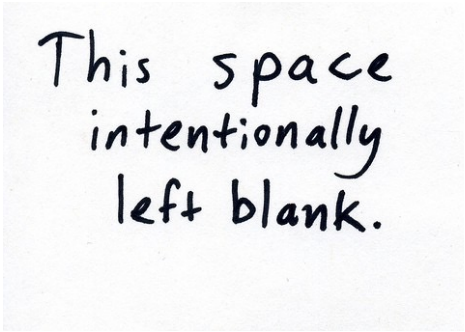
# Introduction: Predictive methods

Intrinsic / *Ab-initio*

Extrinsic

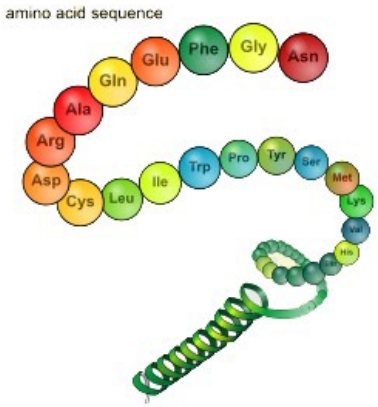
## Ø

- Use of information/features from the sequence itself



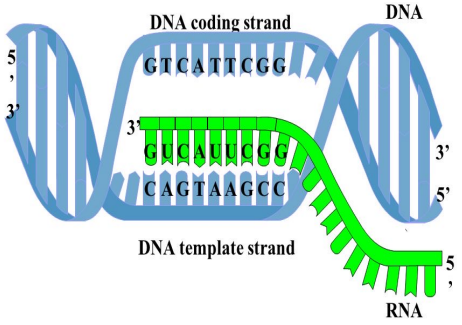

## Proteins

- Known amino acid sequences from other organisms



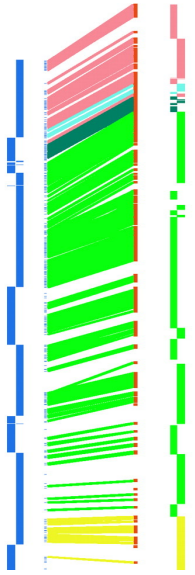

## Transcripts

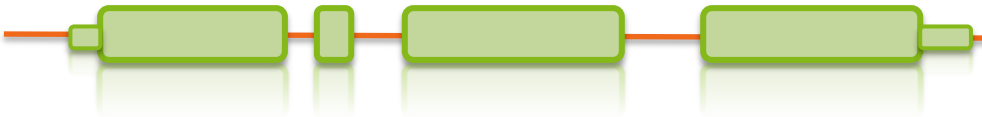
- Assembled from RNA-seq or downloaded ESTs




## Genomes

- Close relative genomes





# 2. The different methods/approaches

# The different approaches

- Similarity-based methods :

  These use similarity to annotated sequences like proteins, cDNAs, or ESTs

- *Ab initio* prediction :

  Likelihood based methods

- Hybrid (*Ab initio* evidence-driven) approaches :

  *Ab initio* tools with the ability to integrate external evidence/hints

- Comparative (homology) based gene finders :

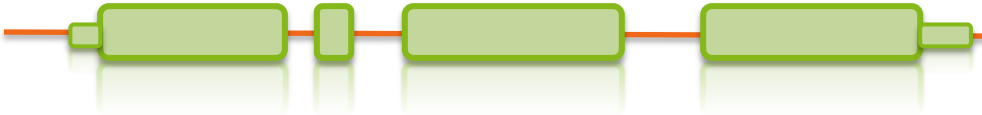
  These align genomic sequences from different species and use the alignments to guide the gene predictions

- Chooser, combiner approaches :

  These combine gene predictions of other gene finders

- Pipelines :

  These combine multiple approaches



# 2.1. Extrinsic approaches
## Similarity-based methods

These use similarity to annotate sequences like

- Proteins
- Transcripts
- ESTs

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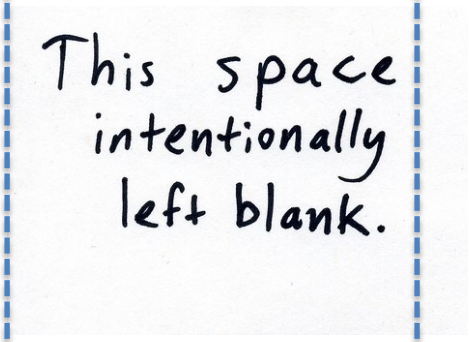
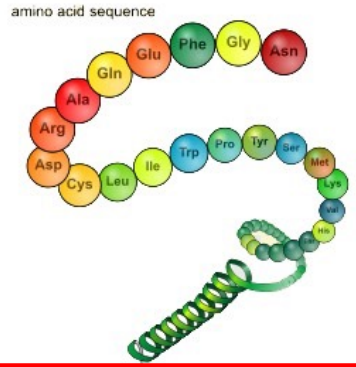
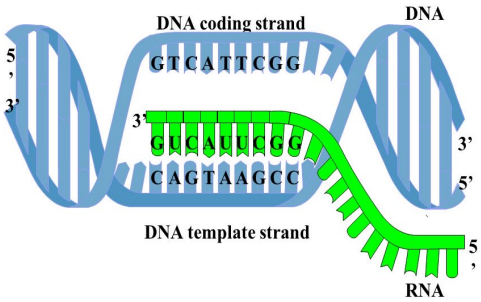
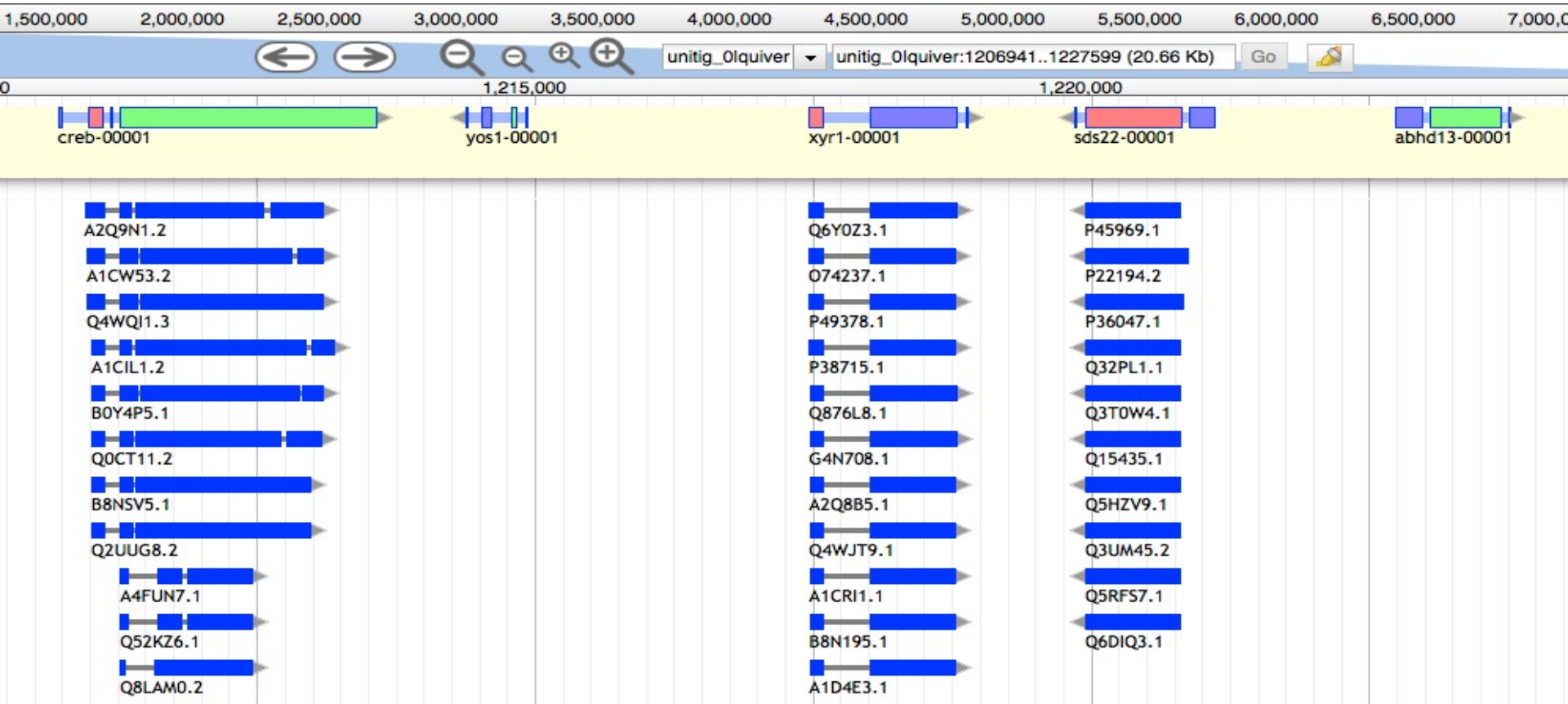
# Types data used vs methods

| Annotation approach | ∅ | Proteins<br>Known amino acid sequences from other organisms | Transcripts<br>Assembled from RNA-seq or downloaded ESTs |
|---|---|---|---|
| |  |  |  |
| Similarity | | X | X |
| Pure ab initio | X | | |
| Hybrid | X | X | X |
| Comparative | X | X | X |
| Chooser/combiner | X | X | X |
| Pipeline | X | X | X |

# Similarity-based method: Protein data

Protein sequences are aligned to the genome

- Conserved in sequence => conserved annotation with little noise



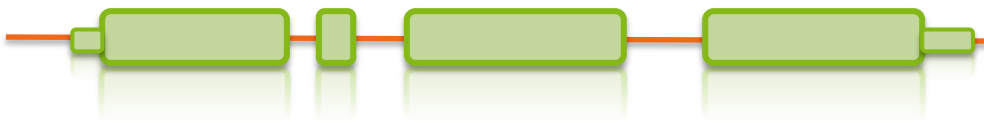
# Similarity-based method: Protein data

**Limits :**

- Related to pre-existing data
- Proteins from model organisms often used => bias?
- Proteins can be incomplete
- Protein can be wrong (PE)
- No UTR

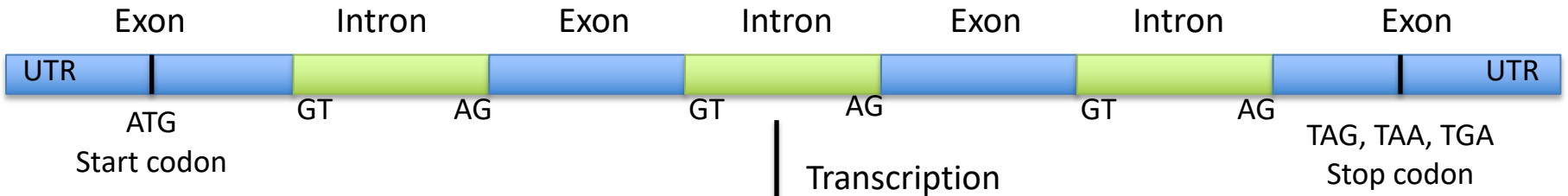
>sp|Q9NSK7|CS012_HUMAN Protein C19orf12 OS=Homo sapiens OX=9606 GN=C19orf12 **PE=1** SV=3
MERLKSHKPATMTIMVEDIMKLLCSLSGERKMKAAVKHSGKGALVTGAMAFVGGLVGGPP
GLAVGGAVGGLLGAWMTSGQFKPVPQILMELPPAEQQRLFNEAAAIIRHLEWTDAVQLTA
LVMGSEALQQQLLAMLVNYVTKELRAEIQYDD

>sp|Q2V2T9|SCL16_ARATH Putative scarecrow-like protein 16 OS=Arabidopsis thaliana OX=3702 GN=SCL16 **PE=5** SV=1
MQIPTLIDSMANKLHKKPPPLLKLTVIASDAEFHPPPLLGISYEELGSKLVNFATTRNVA
MEFRIISSSYSDGLSSLIEQLRIDPFVFNEALVVNCHMMLHYIPDEILTSNLRSVFLKEL
RDLNPTIVTLIDEDSDFTSTNFISRLRSLYNYMWIPYDTAEMFLTRGSEQRQWYEADISW
KIDNVVAKEGAERVERLEPKSR

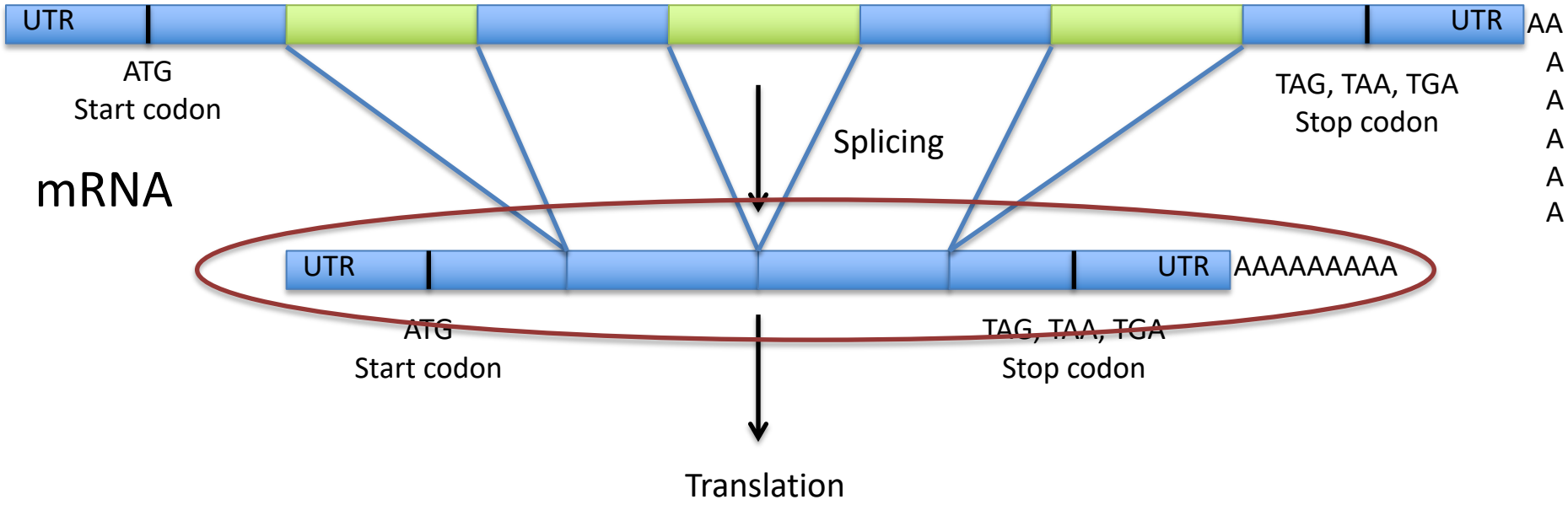


# Types of data used: RNA-seq

## DNA




## Pre-mRNA



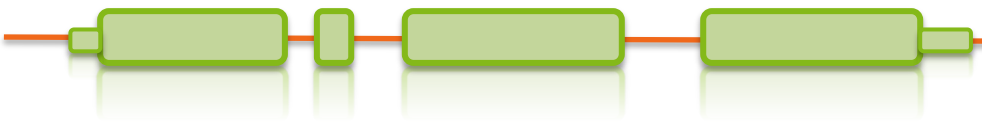
# Similarity-based method: RNA-seq data

- From the same organism as the genomic data => unbiased
- Sample different tissues or life stages if possible

- short-reads:
  - need to be assembled first
    - Genome guided assembly
      ⇒ Stringtie: mapped reads -> transcripts
    - *De novo*
      => Trinity: assembles transcripts without a genome

- Long-reads: IsoSeq …

# Similarity-based method: RNA-seq data



**Limits :**

/!\ Can be very noisy (tissue/species dependent), can include pre-mRNA

=> Avoid gonads and brain ; muscle is good

Hard to catch low expressed / peculiar expressed (stage of life, condition, etc…) / isoforms

- short-reads:
  - Transcriptome assembly errors
- Long-reads:
  - error rate / frameshift / indels

# Similarity-based method: Protein / transcripts

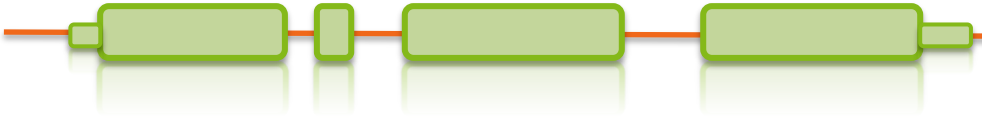
- Rough approximation (fast)

| DNA | AA |
|---|---|
| Blastn | Pmatch |
| Vsearch | tblastn |
| NSimScan | PSimScan |

- Splice-site aware alignment (slow – moderately slow)

| | Method | Human | | | | | |
|---|---|---|---|---|---|---|---|
| | | Mouse | | | Chicken | | |
| CDS | EXALIN | 3h | 5min | 41.3s | 2h | 1min | 41.1s |
| | Exonerate | | 9min | 30.1s | | 3min | 31.7s |
| | GeneSeqer | 7h | 14min | 48.2s | 3h | 2min | 49.4s |
| | GMAP | | 1min | 43.5s | | 1min | 37.9s |
| | PairagonX | 274h | 1min | 16.0s | 500h | 57min | 16.8s |
| | sim4cc | | | 33.9s | | | 19.3s |
| | Spaln2TBZX | | 6min | 55.2s | | 9min | 44.3s |
| | SplignX | | 14min | 2.0s | | 6min | 24.6s |
| | XAT | | 2min | 2.8s | | 1min | 17.9s |
| protein | Exonerate | 12h | 36min | 10.3s | 7h | 33min | 17.0s |
| | genBlastG | | 3min | 30.3s | | 2min | 16.6s |
| | GeneSeqer | 10h | 10min | 24.0s | 6h | 20min | 40.0s |
| | GeneWise | 69h | 17min | 36.1s | 47h | 36min | 6.6s |
| | ProSplign | 2h | 18min | 24.9s | 1h | 17min | 39.1s |
| | Spaln2TBZ | | 4min | 32.0s | | 4min | 41.8s |

| DNA | AA |
|---|---|
| Exonerate | Exonerate |
| Gmap | Genewise |
| GenomeThreader | GenomeThreader |

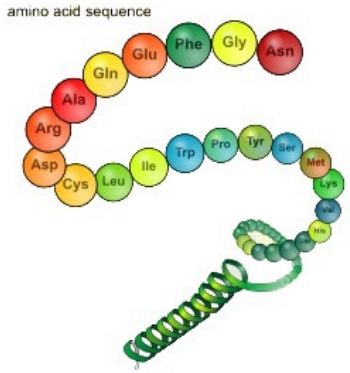
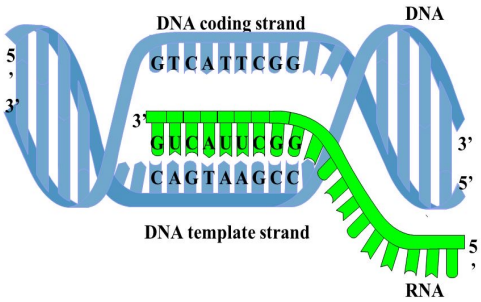


# 2.2 Intrinsic / *ab initio*

Using a probabilistic models to predict features

# The different approaches

## Types data used vs methods

| Annotation approach | ∅ | Proteins<br>Known amino acid sequences from other organisms | Transcripts<br>Assembled from RNA-seq or downloaded ESTs |
|---|---|---|---|
| | This space intentionally left blank. |  |  |
| Similarity | | X | X |
| Pure ab initio | X | | |
| Hybrid | X | X | X |
| Comparative | X | X | X |
| Chooser/combiner | X | X | X |
| Pipeline | X | X | X |

# How it works?

method based on **gene content :**
(statistical properties of protein-coding sequence )

- codon usage
- hexamer usage
- GC content
- compositional bias between codon positions
- nucleotide periodicity
- exon/intron size
- …

and on **signal detection**:

- Promoter
- ORF
- Start codon
- Splice site (Donor and acceptor)
- Stop codon
- Poly(A) tail
- CpG islands
- …

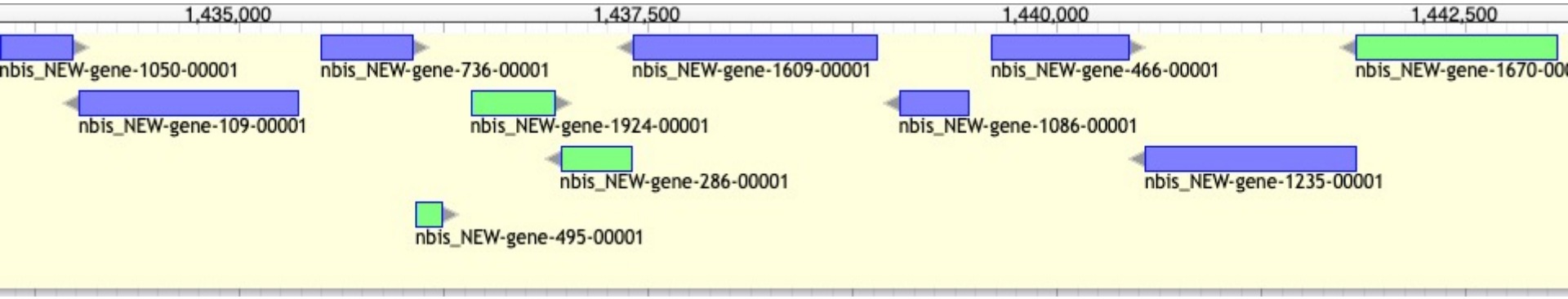
=> *Ab initio* tools will combine this information through different Probabilistic models: HMM, GHMM, WAM, etc.

These models need to be created if not already existing for your organism => **training!**

# Intrinsic / *ab initio*

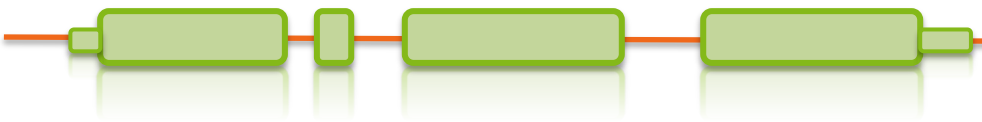
- Bacterial gene prediction

  - Short intergenic regions
  - Uninterrupted ORFs (No intron)
  - Very conserved signals




$\Rightarrow$ easy problem: Accuracy > 90%

# Intrinsic / *ab initio*



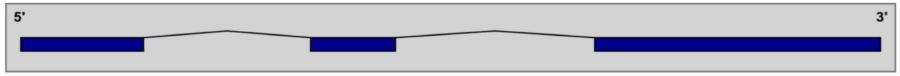
- Eukaryotic gene prediction

  - Presence of intron => structures Differ
  - Isoforms
  - New features (e.g. lncRNAs)

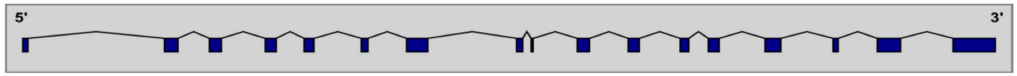
Ostreococcus


Populus


Spruce




Ectocarpus




⇒ Hard problem: Accuracy < 70%

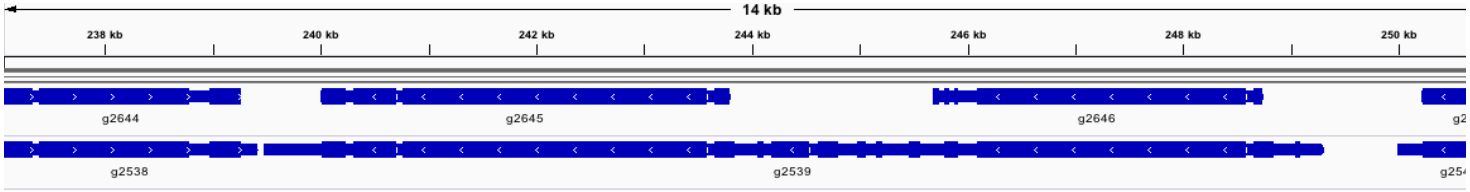
⇒ **Requires training**

# Training *ab-initio* gene-finders

- *Ab-initio* tools need a probabilistic model (also called profile) => Training
  - Few self-trained tools, most need a separate training procedure
  - The quality of the gene-finder results, hugely, relies on the quality of the training!
  - Needs training for every genome (= different training sets)

A fungal genome

Fungi
Plants

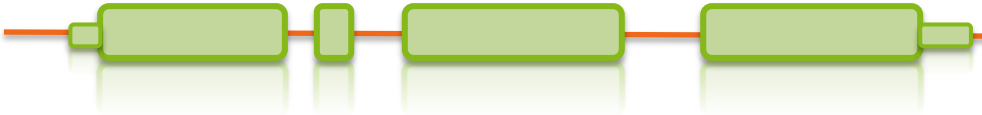



Training:

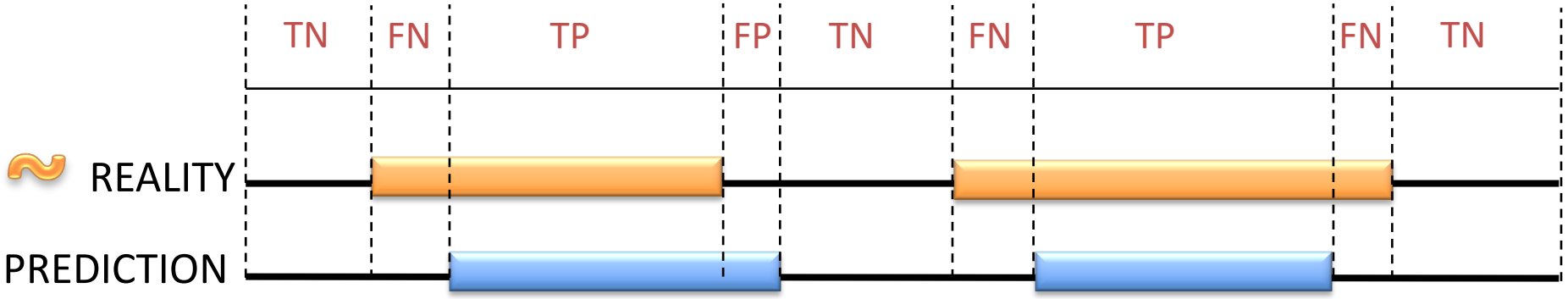
- sets of high quality genes (>500)

  => These ”known” genes are usually inferred from aligned transcripts or proteins

# Intrinsic / *ab initio*



Assess the quality of the *ab-initio* model/training:




**Sensitivity** is the proportion of true predictions compared to the total number of correct genes (including missed predictions)

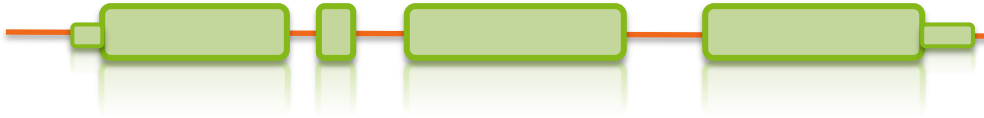
$$Sn = \frac{TP}{TP+FN}$$

**Specificity** is calculated as the number of correct negative predictions divided by the total number of negatives.

$$Sp = \frac{TN}{FP+TN}$$

*Ab Initio* methods can approach 100% sensitivity, however as the sensitivity increases, accuracy suffers as a result of increased false positives.

# Intrinsic / *ab initio*



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******* Evaluation of gene prediction *******

| | sensitivity | specificity |
|---|---|---|
| nucleotide level | 0.987 | 0.896 |

| | #pred total/ unique | #anno total/ unique | TP | FP = false pos. | | | FN = false neg. | | | sensitivity | specificity |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | part | ovlp | wrng | part | ovlp | wrng | | |
| exon level | 512 | 472 | 427 | 29 | 2 | 85 | 30 | 1 | 45 | 0.905 | 0.834 |
| | 512 | 472 | | | | 54 | | | 14 | | |

| transcript | #pred | #anno | TP | FP | FN | sensitivity | specificity |
|---|---|---|---|---|---|---|---|
| gene level | 105 | 100 | 67 | 38 | 33 | 0.67 | 0.638 |

# Intrinsic / *ab initio*

**Popular tools:**

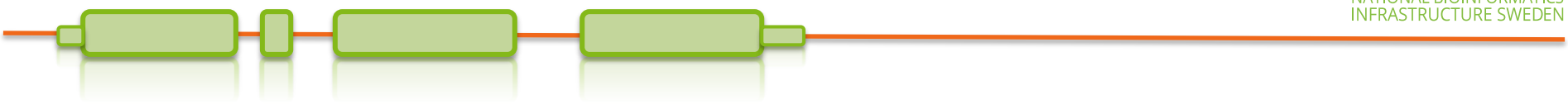
- **SNAP** Works ok, easy to train, not as good as others especially on longer intron genomes.
- **Augustus** Works great, hard to train (but getting better).
- **GeneMark-ES** Self training, no hints, buggy, not good for fragmented genomes or long introns (Best suited for Fungi).
- **FGENESH** Works great, costs money even for training.

Supported by MAKER

http://weatherby.genetics.utah.edu/MAKER/wiki/index.php/MAKER_Tutorial

- **GlimmerHMM** (Eukaryote)
- **GenScan**
- **Gnomon** (NCBI)

# Intrinsic / *ab initio*



**Strengths :**

- Fast and easy
- Annotate unknown genes
- Sensitivity ok
- Need no external evidence

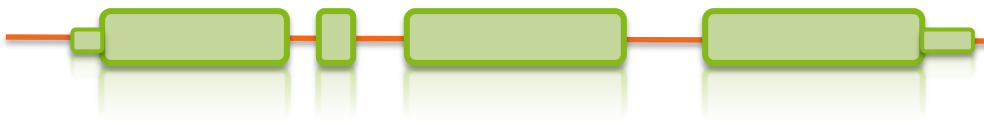
**Limits :**

- No UTR
- No alternatively spliced transcripts

- Bad specificity (Over prediction of exons or/and genes)
- **Training** needed (Need external evidence)

**Common errors in annotation:**

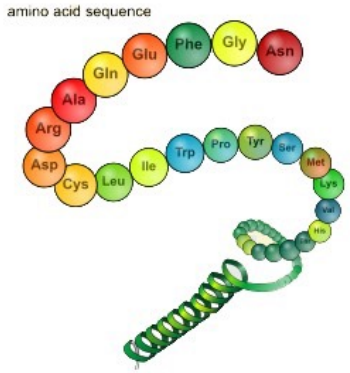
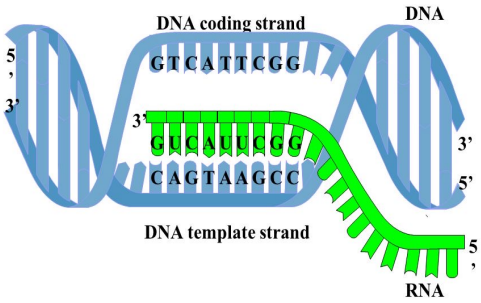
- Split single gene into multiple predictions
- Fused with neighboring genes
- Less accurate than homology based method:
  - Exon boundaries
  - Splicing sites

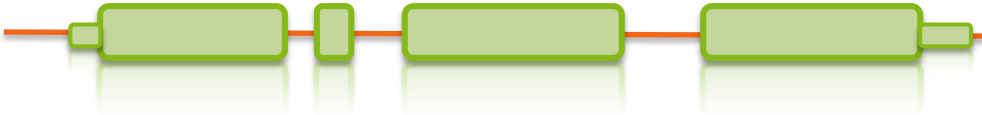


# 2.3 Hybrid approaches
# (*Ab initio* evidence-driven)

*Ab initio* tools with the ability to integrate external evidence/hints

# Types data used vs methods

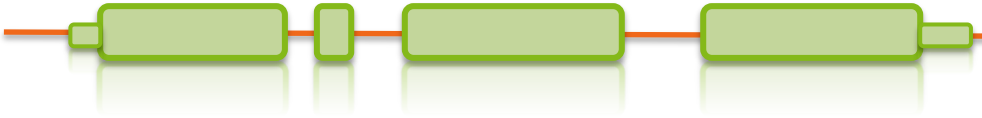
| Annotation approach | ∅ | Proteins<br>Known amino acid sequences from other organisms | Transcripts<br>Assembled from RNA-seq or downloaded ESTs |
|---|---|---|---|
| | This space intentionally left blank. |  |  |
| Similarity | | X | X |
| Pure ab initio | X | | |
| Hybrid (*Ab initio* evidence-driven) | X | X | X |
| Comparative | X | X | X |
| Chooser/combiner | X | X | X |



***Evidence-drivable gene predictors*** approaches incorporate hints/extrinsic information in the form of alignments (transcript, protein, whole genome) to increase the accuracy of the gene prediction.

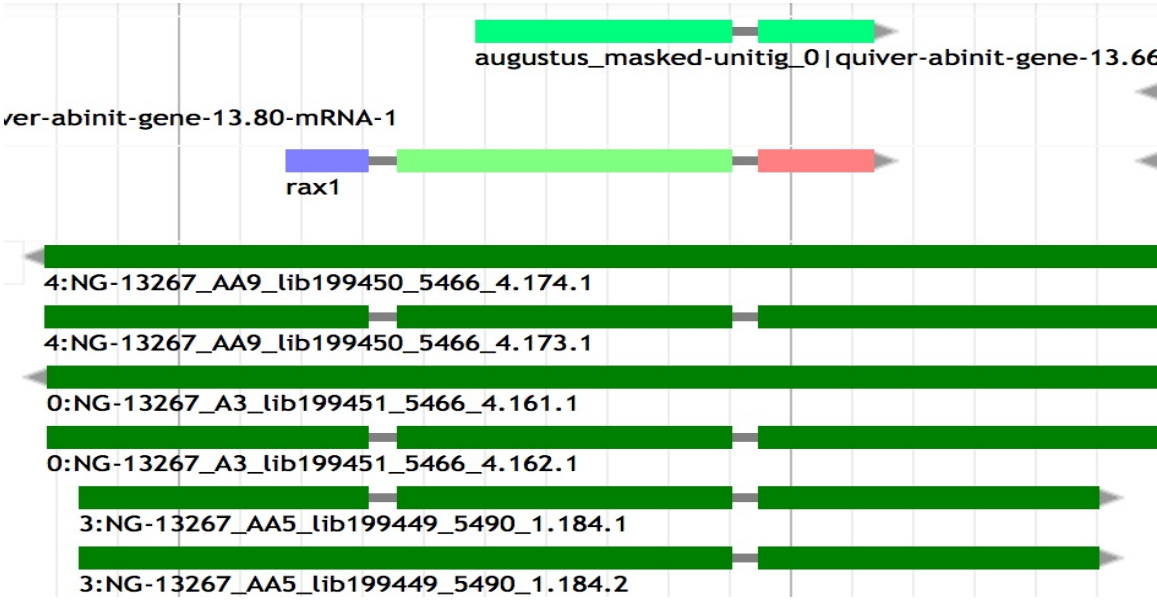
- Can predict locus without extrinsic information
- Improve prediction when extrinsic information available

# *Hybrid : Ab initio* evidence-driven



**Strength :**

- Lot of data available
- Protein well conserved
- High accuracy




**Limits :**

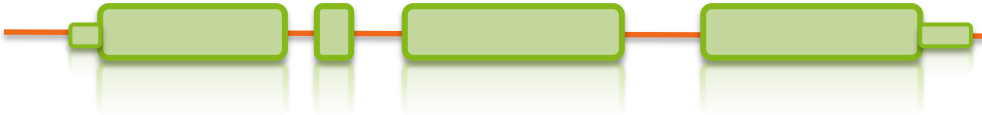
- **Extra computation to generate alignments**
- **heterogeneous sequence quality** :
  - Incomplete
  - Error during transcriptome assembly
  - Contamination
  - Sequence missing
  - Orientation error

## Tools

- **GenomeScan** Blast hit used as extra guide
- **Augustus** 16 types of hints accepted (gff): start, stop, tss, tts, ass, dss, exonpart, exon, intronpart, intron, CDSpart, CDS, UTRpart, UTR, irpart, nonexonpart.
- **GeneMark-ET** EST-based evidence hints
- **GeneMark-EP** Protein-based evidence hints } Self training !
- **SNAP** Accepts EST and protein-based evidence hints.
- **Gnomon*** Uses EST and protein alignments to guide gene prediction and add UTRs
- **FGENESH+** Best suited for plant
- **EuGene*** Any kind of evidence hints. Hard to configure (best suited for plant)

* Can be seen as combiner

# *Hybrid : Ab initio* evidence-driven



The BRAKER1 gene finding pipeline:

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

Katharina J. Hoff et *al.*

Bioinformatics (2016) 32 (5): 767-769. doi: 10.1093/bioinformatics/btv661

- BRAKER1 was more accurate than MAKER2 when it is using RNA-Seq as sole source for training and prediction.
- BRAKER1 does not require pre-trained parameters or a separate expert-prepared training step.

**BRAKER2 since 2019** (Incorporate Protein Homology Information)

# 2.4 Comparative based method

These align genomic sequences from different species and use the alignments to guide the gene predictions

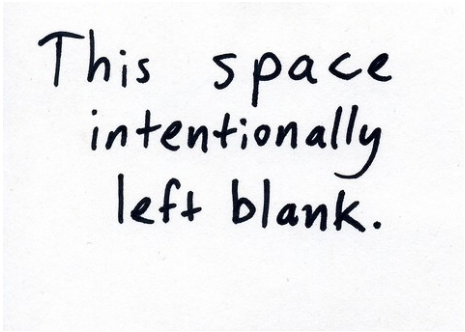
# The different approaches

Intrinsic / *Ab-initio*

Extrinsic

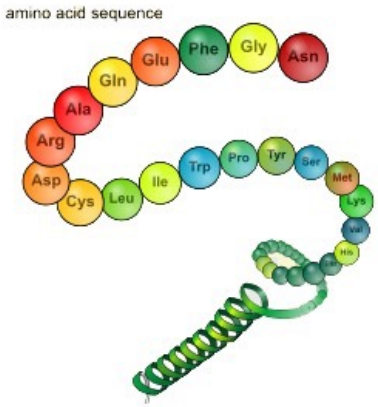
## Ø

- Use of information/features from the sequence itself



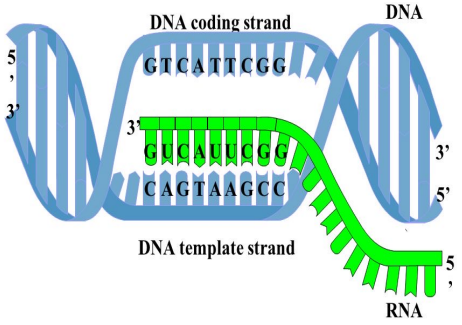

## Proteins

- Known amino acid sequences from other organisms



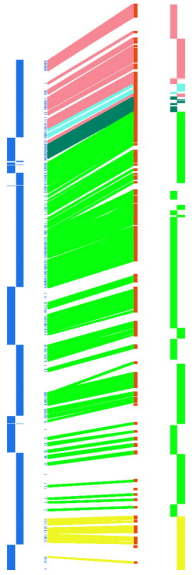

## Transcripts

- Assembled from RNA-seq or downloaded ESTs



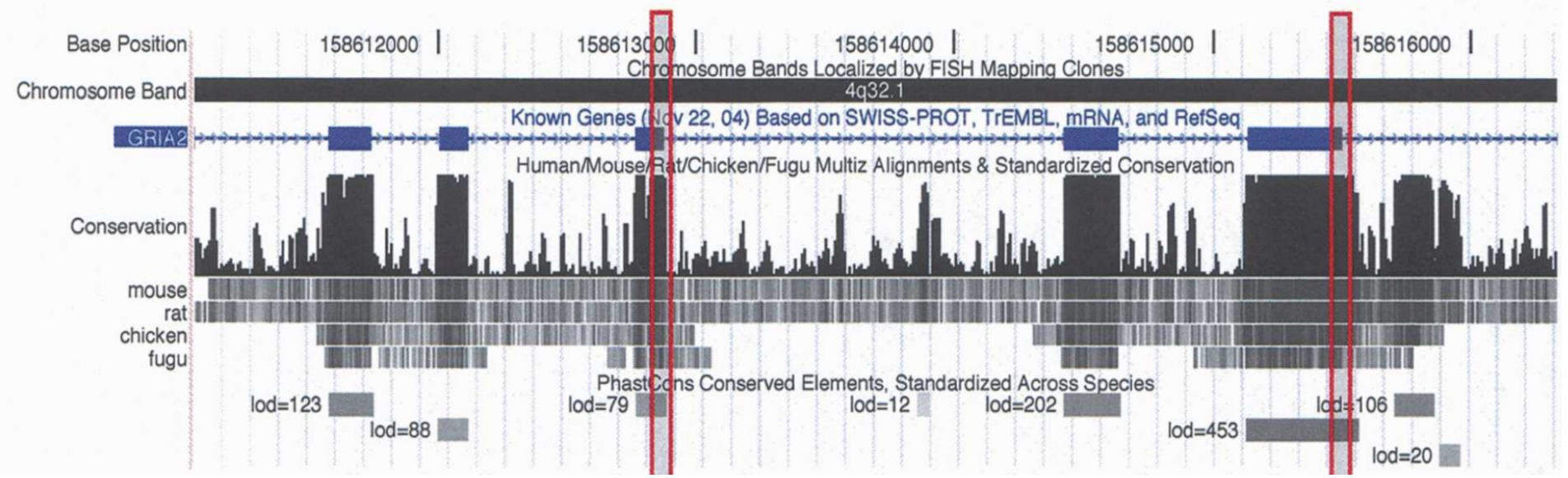

## Genomes

- Close relative genomes



# Comparative-based method

The main assumption of these methods is that the functional parts of an eukaryotic genomic sequence, the exons, tend to be more conserved than the non-functional ones, the introns.




These align genomic sequences from different species and use the alignments to guide the ab-initio gene predictions.

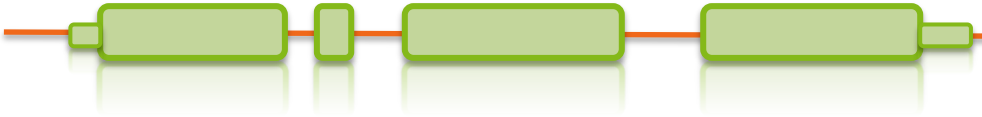
**Limits :** - Whole genome alignment is time/memory consuming

- Need relatively close related genome (<50 My)

# Comparative-based method: Tools

- **Dual genome**, de novo gene structure prediction:
  - **RosettaCM** (Pioneer – 2000)
  - **SGP-2** (2001) – considers only the conservation in protein-coding regions
  - **TWINSCAN** (2001) - included models of conservation in splice sites and start and stop codons
  - **SLAM** (2003)
  - **TWAIN** (2005)
- More than 2 genomic sequences:
  - **NSCAN*** (2006)
  - **Conrad** *(CRF, 2007)
  - **CONTRAST*** (CRF, 2008) -> 58% accuracy
  - **GSA-MPSA**
  - **Augustus-CGP***

*Can use similarity-based information



# 2.5 Combiner / Chooser

- *Combining heterogeneous data into gene models*
- *Selection of gene models*

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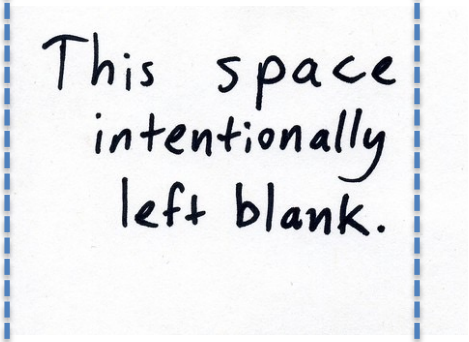
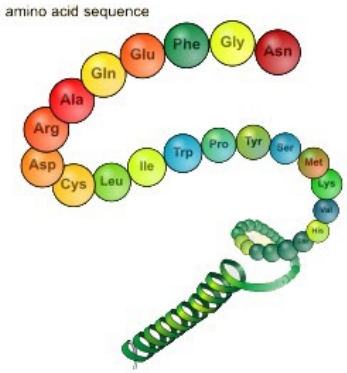
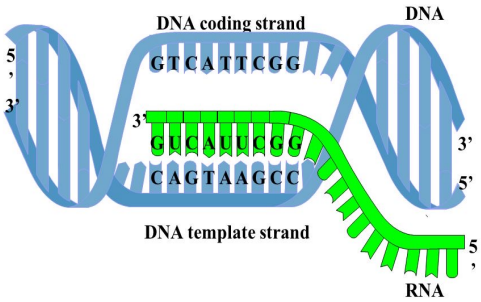
# Types data used vs methods

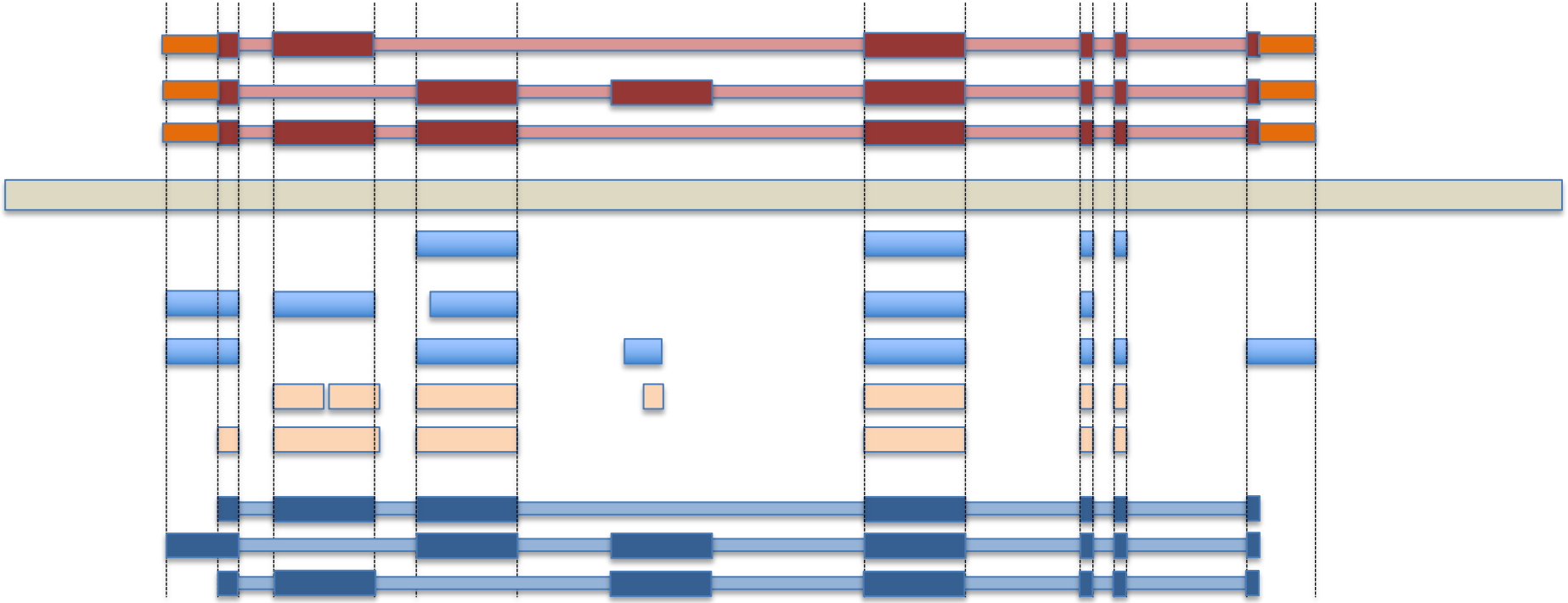
| Annotation approach | ∅ | Proteins<br>Known amino acid sequences from other organisms | Transcripts<br>Assembled from RNA-seq or downloaded ESTs |
|---|---|---|---|
| |  |  |  |
| Similarity | | X | X |
| Pure ab initio | X | | |
| Hybrid | X | X | X |
| Comparative | X | X | X |
| Chooser/combiner | X | X | X |
| Pipeline | X | X | X |

# Chooser / combiner

**Combiner concept:** combining different lines of evidence into gene models

Evidence: ESTs / Transcripts Proteins
Gene prediction (*ab-initio* or evidence-based)
Gene models

**=> add untranslated regions (UTR)**



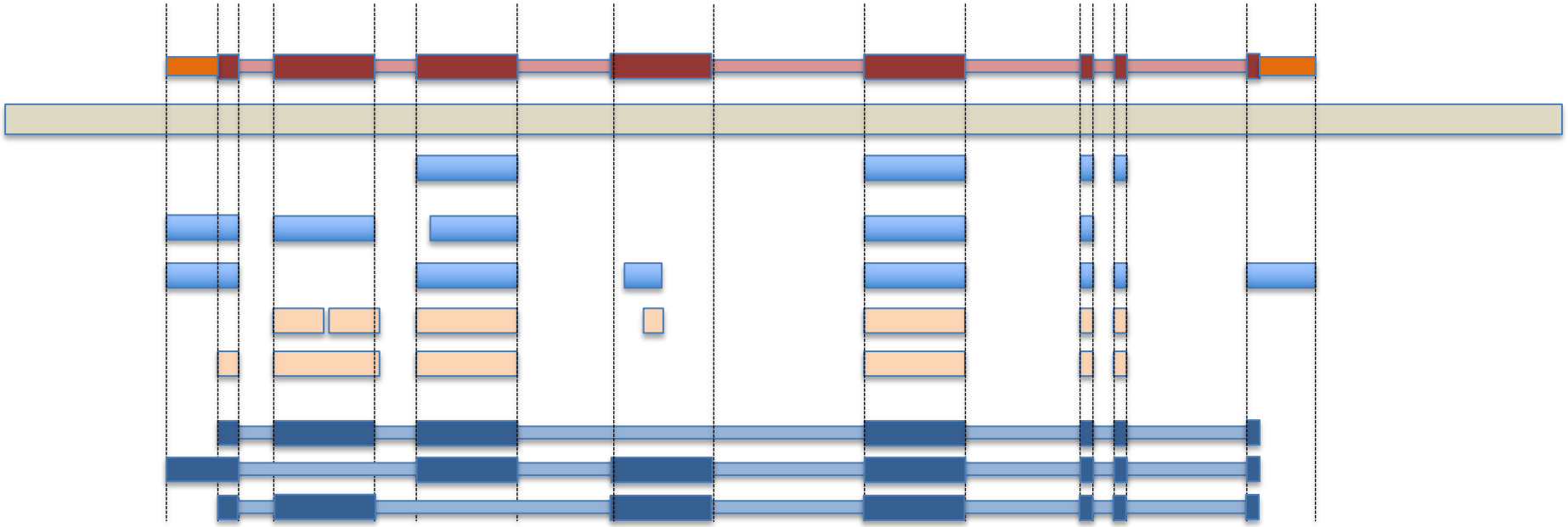
# Chooser / combiner

**Combiner concept:** combining different lines of evidence into gene models

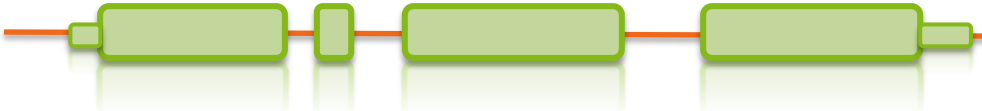
Evidence: ESTs / Transcripts / Proteins

Gene prediction (*ab-initio* or evidence-based)

**=> Select the best possible set of exons and combine them in a consensus gene model**



# Chooser / combiner

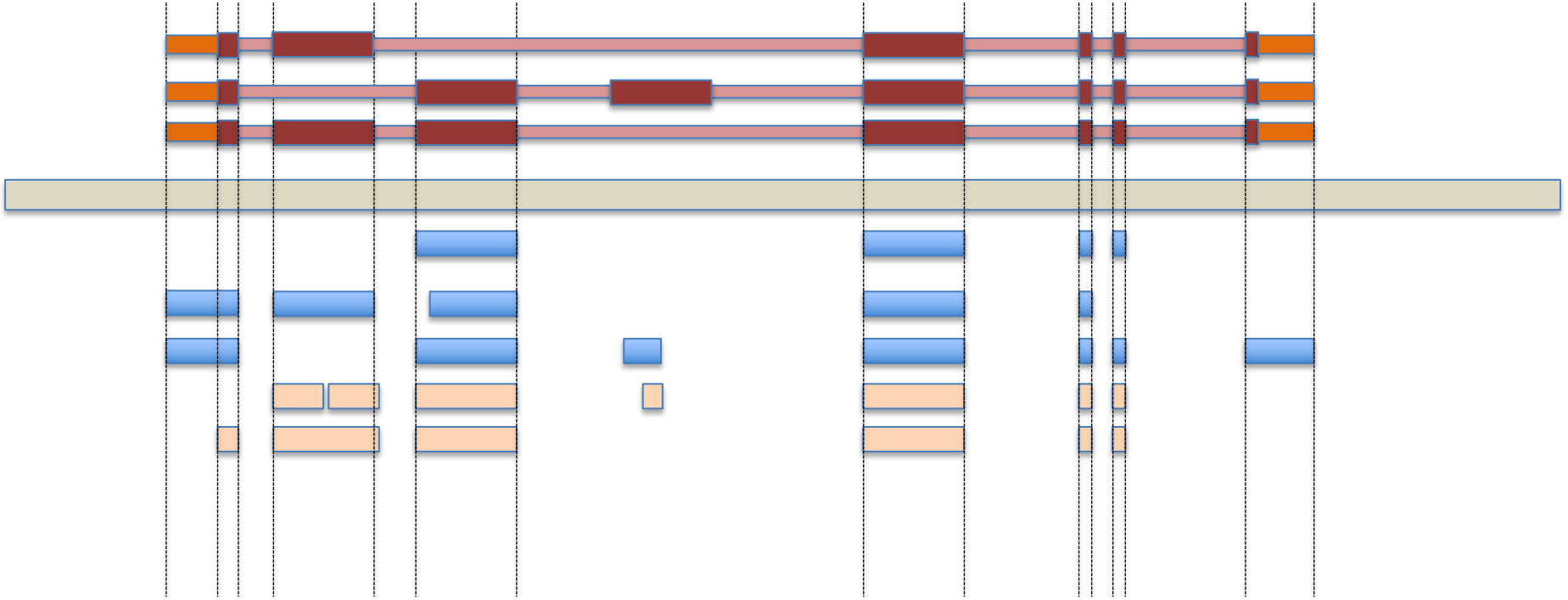


**Chooser concept:** Select best gene models

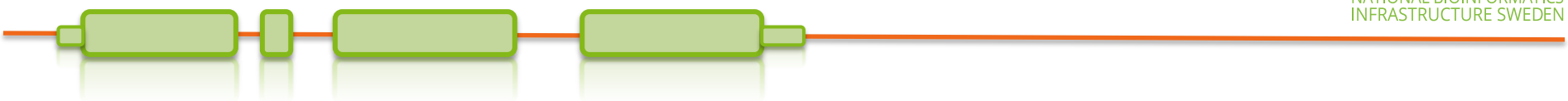
Evidence: ESTs / Transcripts / Proteins

Gene models

**=> Choose the prediction whose best matches the evidence**



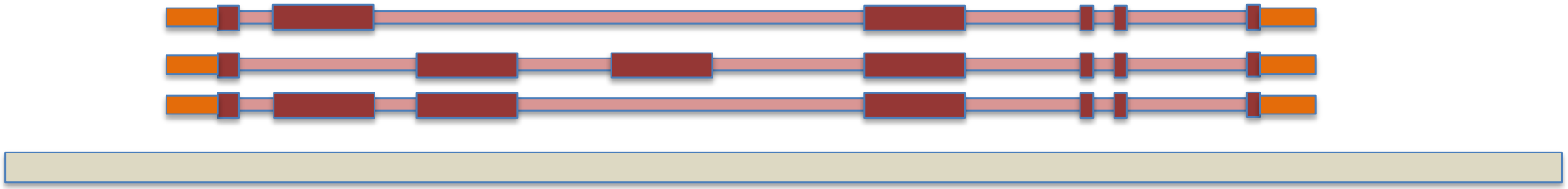
# Chooser / combiner



**Chooser concept:** Select best gene models

Gene models

**=> Choose the prediction whose structure best represents the consensus**



# Chooser / combiner

**Use gene finders and evidence (EST, RNAseq, protein) alignments and:**

| Tool | Consensus based chooser | Evidence based chooser | weight of different sources | Comment |
|---|---|---|---|---|
| A) Choose the prediction whose best matches the evidence | | | | |
| **MAKER*** | | X | | |
| **PASA*** | | X | | |
| B) Choose the prediction whose structure best represents the consensus | | | | |
| **JIGSAW** | X | | | |
| C) Choose the best possible set of exons and combine them in a gene model | | | | |
| **EVM** Evidencemodeler | X | X | X | User can set the expected evidence error rate manually or/and learn from a training set |
| **Evigan** | X | | X | Unsupervised learning method |
| **Ipred** | | X | | Does not require any a priori knowledge<br>Can also combine only evidences to create a gene model |

**Strength =>** They improve on the underlying gene prediction models

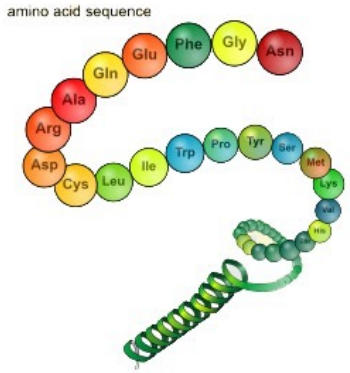
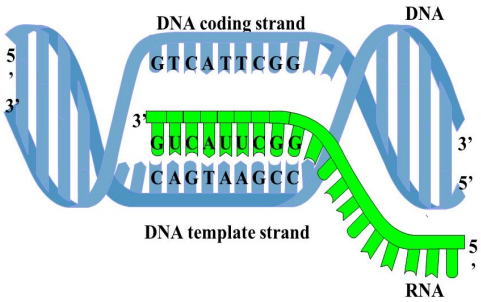
* Are pipelines that contain chooser

# 2.6 Pipelines

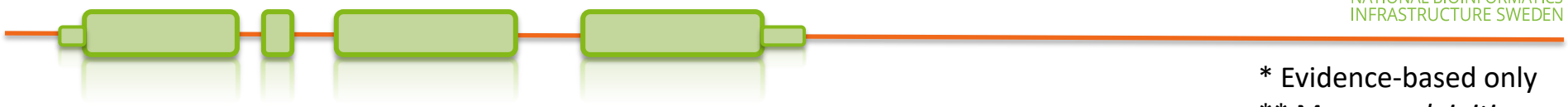
(The ultimate step)

- *Align evidence*
- *add annotation (UTR, score, gene name)*
- *Annotation of other features (Repeat, tRNA, etc)*
- *And more…*

# Types data used vs methods

| Annotation approach | ∅ | Proteins<br>Known amino acid sequences from other organisms | Transcripts<br>Assembled from RNA-seq or downloaded ESTs |
|---|---|---|---|
| | This space intentionally left blank. |  |  |
| Similarity | | X | X |
| Pure ab initio | X | | |
| Hybrid | X | X | X |
| Comparative | X | X | X |
| Chooser/combiner | X | X | X |
| Pipeline | X | X | X |

# Annotation pipeline



* Evidence-based only
** May use *ab initio*

**PASA*** Produces evidence-driven consensus gene models

- \- minimalist pipeline ()
- \+ good for detecting isoforms
- \+ biologically relevant predictions

=> using *Ab initio* tools and combined with **EVM** it does a pretty good job !

- \- PASA + Ab initio + EVM not automatized

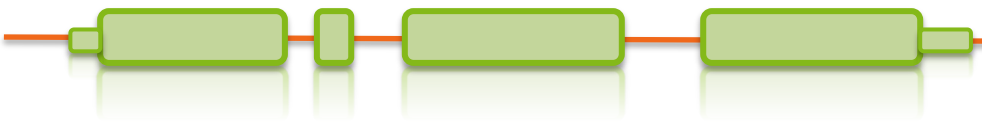
**NCBI pipeline** Evidence + *ab initio* (Gnomon), repeat masking, gene naming, miRNAs, tRNAs, …

**Ensembl**** Evidence based only ( comparative + homology ) …

**Comparative Annotation Toolkit (CAT)** ab-initio (Augustus) evidence driven + comparative

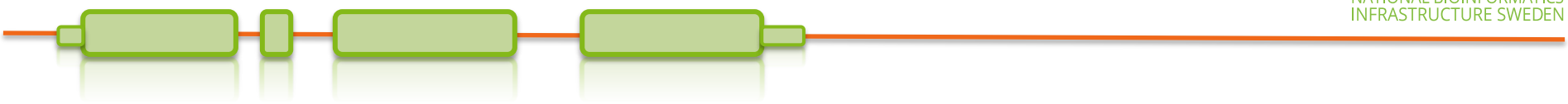
**MAKER2** Evidence based and/or *ab initio* …

…

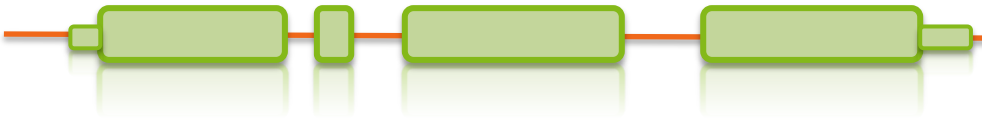


# 3. Annotation of other genome features

# Other genome features



| Feature type | DB associated | Tool example | approach |
|---|---|---|---|
| ncRNA | Rfam | infernal | HMM + CM |
| tRNA | Sprinzl database | tRNAscan-SE | CM + WMA |
| snoRNA | | snoscan | HMM + SCFG |
| miRNA | miRBase | Splign<br><br>miR-PREFeR (for plant) | sequence alignment<br><br>Based on expression patterns |
| Repeats | Repbase, Dfam | repeatMasker | HMM, blast |
| Pseudogenes | | pseudopipe | homology-based (blast) |
| … | | | |



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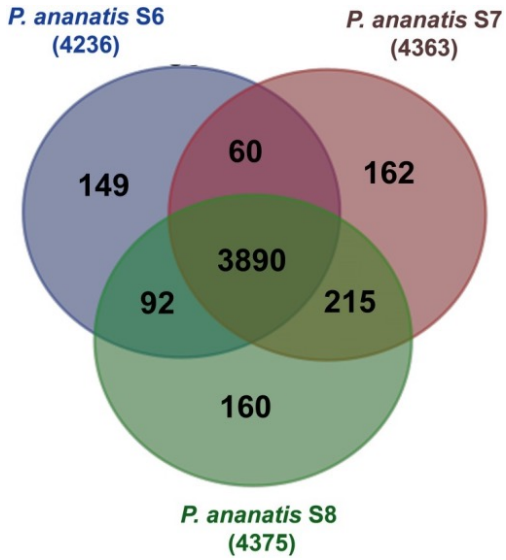
# 4. Assessing an annotation

# Assessing an annotation

- Simple statistics (number genes / number exon per gene)
- BUSCO (and compare against assembly result )
- Protein/transcript evidence (AED score in MAKER)
- Comparative genomics (OrthoMCL)
- Domain / Function attached
- Visualization



# Assessing an annotation

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## Selection of most common visualization or/and Manual curation tools

| Name | Standalone | Web tool | Manual curation | year | comment |
|---|---|---|---|---|---|
| Artemis | X | | X | 2000 | Can save annotation in EMBL format |
| IGV | X | | | 2011 | Popular |
| Savant | X | | | 2010 | Sequence Annotation, Visualization and ANalysis Tool. enable Plug-ins |
| Tablet | X | | X | 2013 | |
| IGB | X | | | 2008 | enable Plug-ins. Can load local and remote data (dropbox, UCSC genome, etc) |
| Jbrowse | | X | | 2010 | GMOD (successor of Gbrowse) |
| Web Apollo | | X | X | 2013 | Active community (gmod). Based on Jbrowse. Real-time collaboration |
| UCSC | | X | | 2000 | A large amount of locally stored data must be uploaded to servers across the internet |
| Ensembl genome browsers | | X | | 2002 | A large amount of locally stored data must be uploaded to servers across the internet |

FOR AN EXHAUSTIVE LIST: https://en.wikipedia.org/wiki/Genome_browser

# 5. To resume / Closing remarks

# Closing remarks

- >100 annotation tools – as many methods (https://github.com/NBISweden/GAAS/blob/master/annotation/knowledge/annotation_tools_genome.md)
- GTF/GFF format
- 6 main class of approaches (Similarity-based, *ab initio*, hybrid, comparative, combiner, pipeline )

**How to choose Method:**

- Scientific question behind ( need of a conservative annotation vs exhaustive)

- Species dependent (plant / Fungi / eukaryotes)

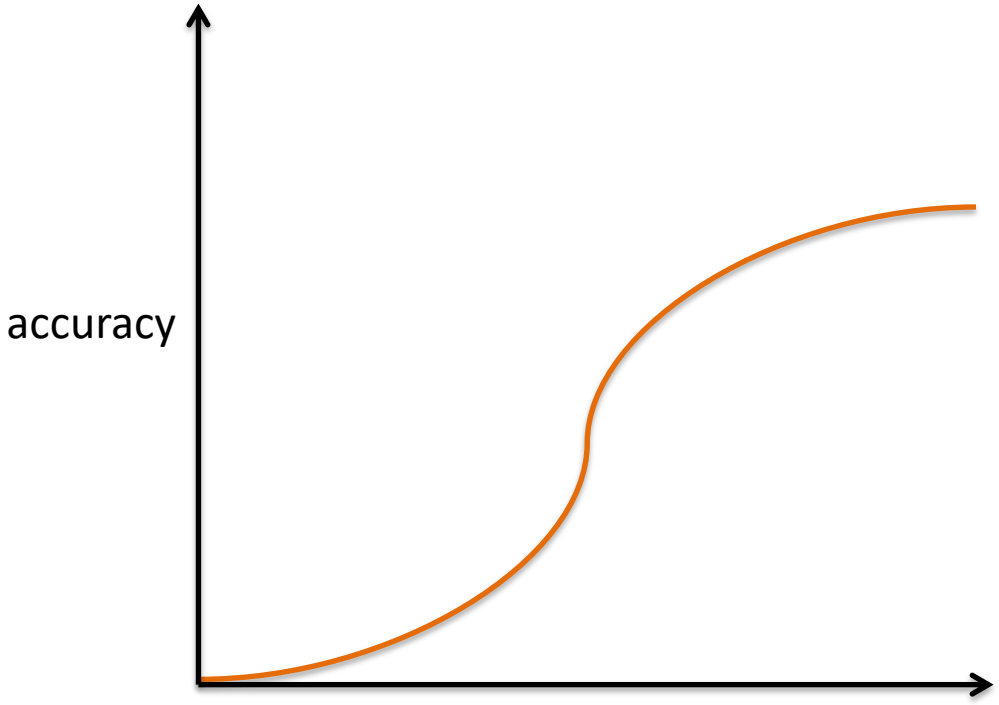
- phylogenetic relationship of the investigated genome to other annotated genomes (Terra incognita, close, already annotated).

- Data available (hmm profile, RNAseq, etc…)

- Depending on computing resources (*ab initio* ~ hours < VS > pipeline ~ weeks)

# Closing remarks

Effort versus accuracy



# Closing remarks

- Several *ab-initio* tools together give better result that one alone
  (they complement each other)

- Pipelines give good results
  MAKER2 the most flexible, adjustable

- Most methods only build gene models, no **functional inference**

- No annotation method is perfect, they make mistakes !!

- Annotation requires **manual curation**

- As for assembly, an annotation is never finished, it can always be improved
  => e.g. Human (to know when to stop)

- Submit your annotation in public archive

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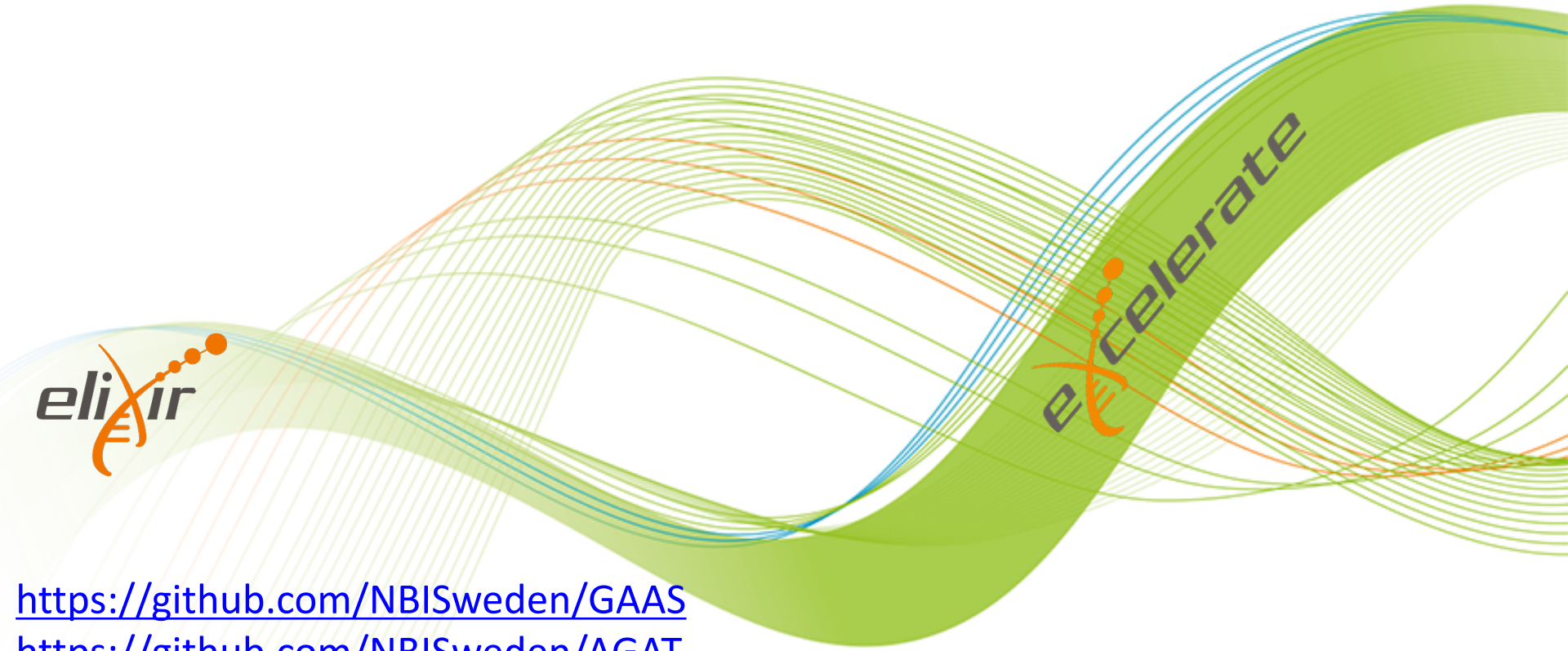
# *THE END*




https://github.com/NBISweden/GAAS
https://github.com/NBISweden/AGAT