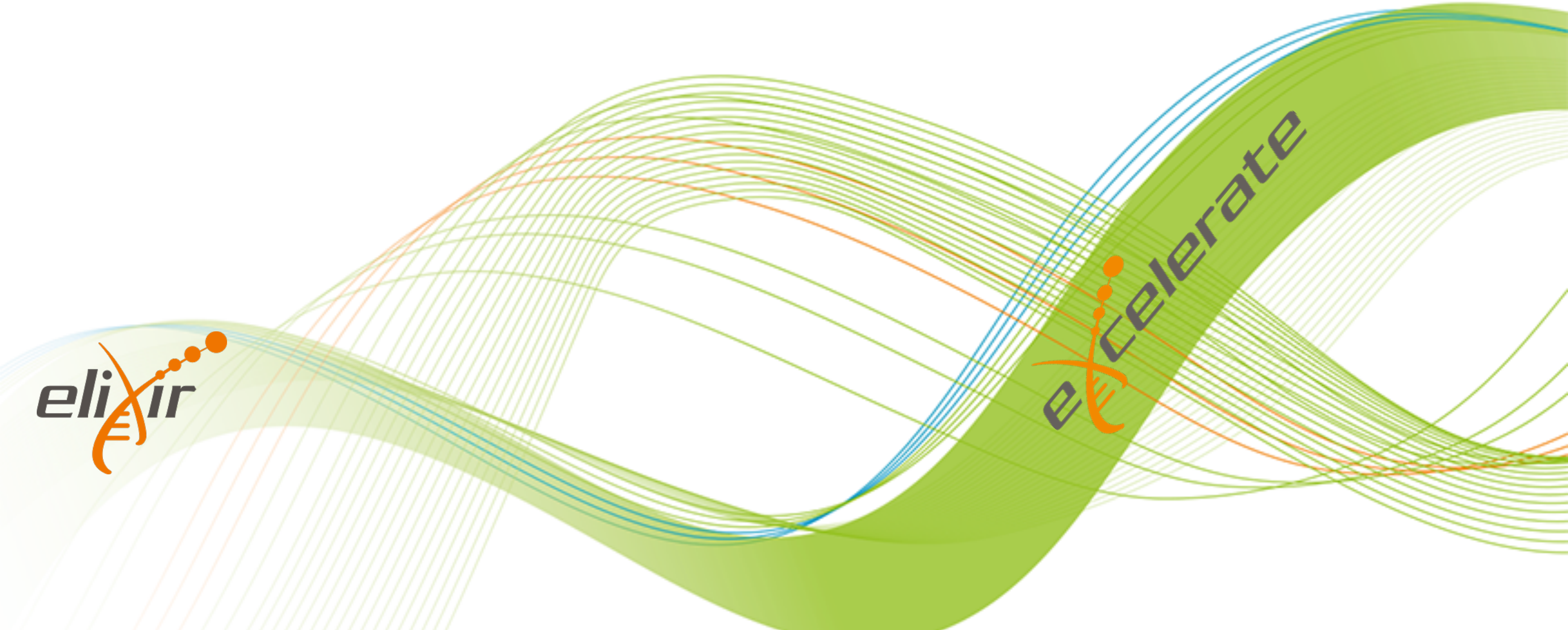
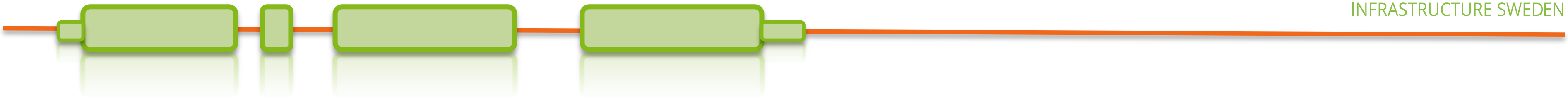
Lucile Soler, PhD
Jacques Dainat, PhD

How to do a genome annotation?



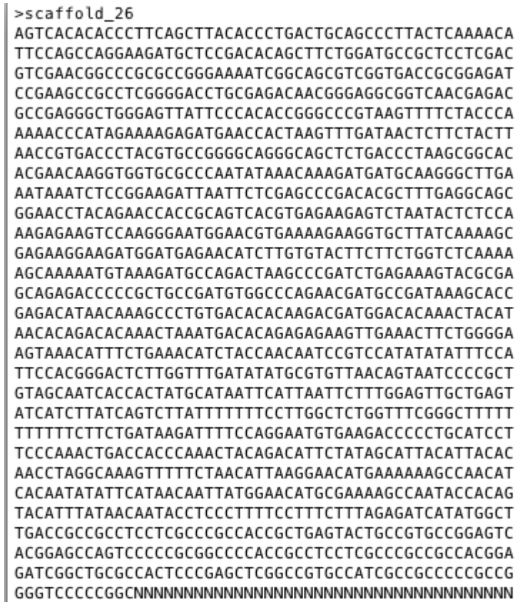
Tromsø – May 2021

Introduction: Formats



From a genome…

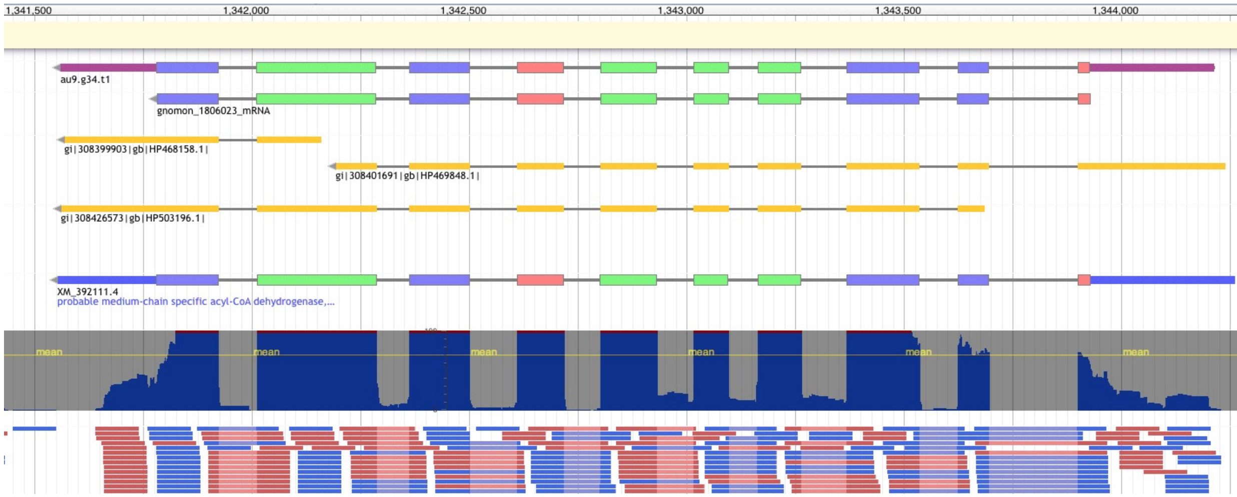
FASTA



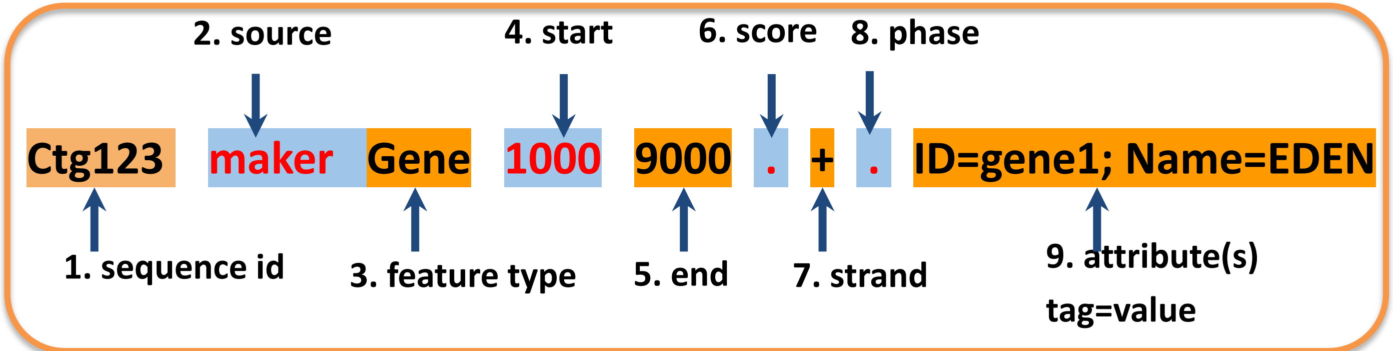
```
>scaffold_26
AGTCACACACCCTTCAGCTTACACCCTGACTGCAGCCCTTACTCAAAACA
TTCCAGCCAGGAAGATGCTCCGACACAGCTTCTGGATGCCGCTCCTCGAC
GTCGAACGGCCCGCGCCGGGAAAATCGGCAGCGTCGGTGACCGCGGAGAT
CCGAAGCCGCCTCGGGGACCTGCGAGACAACGGGAGGCGGTCAACGAGAC
GCCGAGGGCTGGGAGTTATTCCCACACCGGGCCCGTAAGTTTTCTACCCA
AAAACCCATAGAAAAGAGATGAACCACTAAGTTTGATAACTCTTCTACTT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

…to an annotated gene

GTF/GFF


1,341,500
1,342,000
1,342,500
1,343,000
1,343,500
1,344,000
au9.g34.t1
gnomon_1806023_mRNA
gi|308399903|gb|HP468158.1|
gi|308401691|gb|HP469848.1|
gi|308426573|gb|HP503196.1|
XM_392111.4
probable medium-chain specific acyl-CoA dehydrogenase,...
mean
mean
mean
mean
mean
mean

- 9 columns
- 1 feature = 1 line



Ctg123 maker Gene 1000 9000 . + . ID=gene1; Name=EDEN

1. sequence id

2. source

3. feature type

4. start

5. end

6. score

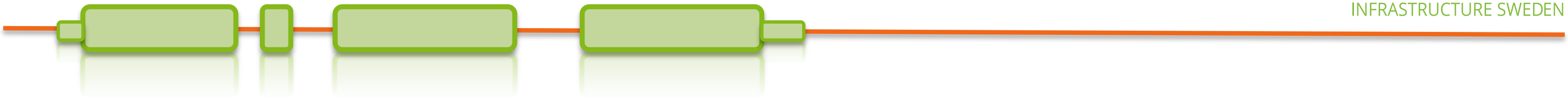
7. strand

8. phase

9. attribute(s)

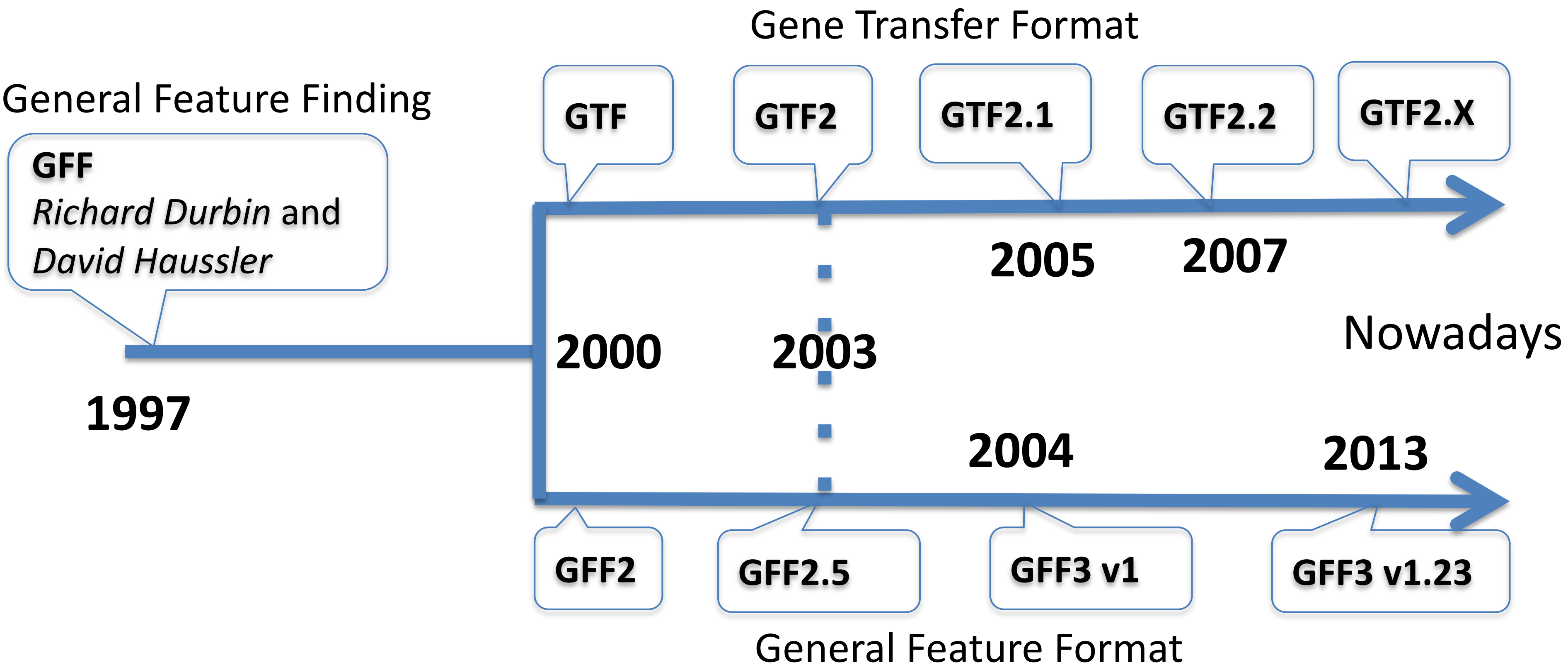
tag=value

Introduction: Formats



❑GFF / GTF formats

https://github.com/NBISweden/GAAS/blob/master/annotation/knowledge/gxf.md



Gene Transfer Format

General Feature Finding

GFF
Richard Durbin and
David Haussler

1997

2000

GTF

GFF2

2003

GTF2

GFF2.5

2004

GFF3 v1

2005

GTF2.1

2007

GTF2.2

GTF2.X

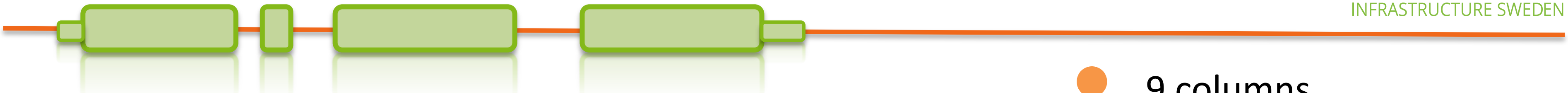
Nowadays

2013

GFF3 v1.23

General Feature Format

Introduction: Formats: GTF2.X



- 9 columns
- 1 feature = 1 line

Header

```
#!genome-build GRCz11
#!genome-date 2017-05
Ctg123  .   Gene        1000  9000  .  +  .  gene_id gene1; name EDEN;
ctg123  .  Transcript   1050  9000  .  +  .  gene_id gene1; transcript_id=t1; name EDEN;
ctg123  .  Transcript   1050  9000  .  +  .  gene_id gene1; transcript_id=t2; name EDEN;
ctg123  .   exon        1300  1500  .  +  .  gene_id gene1; transcript_id=t1; name EDEN;
ctg123  .   exon        1050  1500  .  +  .  gene_id gene1; transcript_id=t1; name EDEN;
tg123   .   exon        1050  1500  .  +  .  gene_id gene1; transcript_id=t2; name EDEN;
ctg123  .   exon        3000  3902  .  +  .  gene_id gene1; transcript_id=t1; name EDEN;
ctg123  .   exon        5000  5500  .  +  .  gene_id gene1; transcript_id=t1; name EDEN;
ctg123  .   exon        5000  5500  .  +  .  gene_id gene1; transcript_id=t2; name EDEN;
ctg123  .   exon        7000  9000  .  +  .  gene_id gene1; transcript_id=t1; name EDEN;
ctg123  .   exon        7000  9000  .  +  .  gene_id gene1; transcript_id=t2; name EDEN;
ctg123  .   CDS         1201  1500  .  +  0  gene_id gene1; transcript_id=t1; name EDEN;
ctg123  .   CDS         3000  3902  .  +  0  gene_id gene1; transcript_id=t1; name EDEN;
ctg123  .   CDS         5000  5500  .  +  0  gene_id gene1; transcript_id=t1; name EDEN;
ctg123  .   CDS         7000  7600  .  +  0  gene_id gene1; transcript_id=t1; name EDEN;
Ctg123  .   CDS         1201  1500  .  +  0  gene_id gene1; transcript_id=t2; name EDEN;
ctg123  .   CDS         5000  5500  .  +  0  gene_id gene1; transcript_id=t2; name EDEN;
Ctg123  .   CDS         7000  7600  .  +  0  gene_id gene1; transcript_id=t2; name EDEN;
```

1) sequence id

2) source

3) feature type
(9 possibilities)

4) start

5) end

6) score

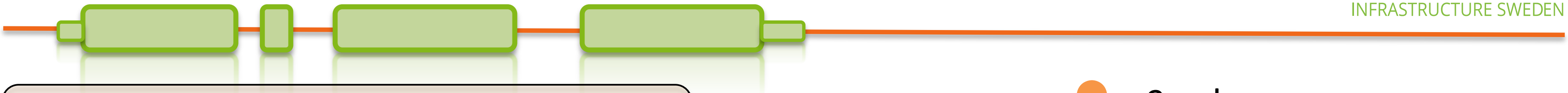
7) strand

8) phase

9) attributes
tag value;

! Features grouped by a **common attribute** (gene_id / transcript_id)

Introduction: Formats: GFF3



```
##gff-version 3.2.1
##sequence-region ctg123 1 1497228
```

Header

- 9 columns
- 1 feature = 1 line

```
Ctg123  .  Gene  1000  9000  .  +  .  ID=gene1;Name=EDEN
ctg123  .  mRNA  1050  9000  .  +  .  ID=mRNA1;Parent=gene1;Name=EDEN.1
ctg123  .  mRNA  1050  9000  .  +  .  ID=mRNA2;Parent=gene1;Name=EDEN.2
ctg123  .  exon  1300  1500  .  +  .  ID=exon1;Parent=mRNA3
ctg123  .  exon  1050  1500  .  +  .  ID=exon2;Parent=mRNA1,mRNA2
ctg123  .  exon  3000  3902  .  +  .  ID=exon3;Parent=mRNA1
ctg123  .  exon  5000  5500  .  +  .  ID=exon4;Parent=mRNA1,mRNA2
ctg123  .  exon  7000  9000  .  +  .  ID=exon5;Parent=mRNA1,mRNA2
ctg123  .  CDS   1201  1500  .  +  0  ID=cds1;Parent=mRNA1;Name=eden1
ctg123  .  CDS   3000  3902  .  +  0  ID=cds1;Parent=mRNA1;Name=eden1
ctg123  .  CDS   5000  5500  .  +  0  ID=cds1;Parent=mRNA1;Name=eden1
ctg123  .  CDS   7000  7600  .  +  0  ID=cds1;Parent=mRNA1;Name=eden1
Ctg123  .  CDS   1201  1500  .  +  0  ID=cds2;Parent=mRNA2;Name=eden2
ctg123  .  CDS   5000  5500  .  +  0  ID=cds2;Parent=mRNA2;Name=eden2
Ctg123  .  CDS   7000  7600  .  +  0  ID=cds2;Parent=mRNA2;Name=eden2
```

1) sequence id

2) source

3) feature type
(SO term = 2278 possibilities)

4) start

5) end

6) score

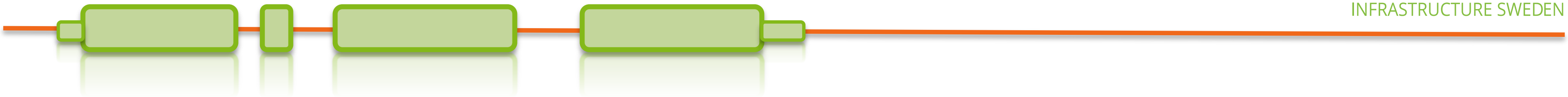
7) strand

8) phase

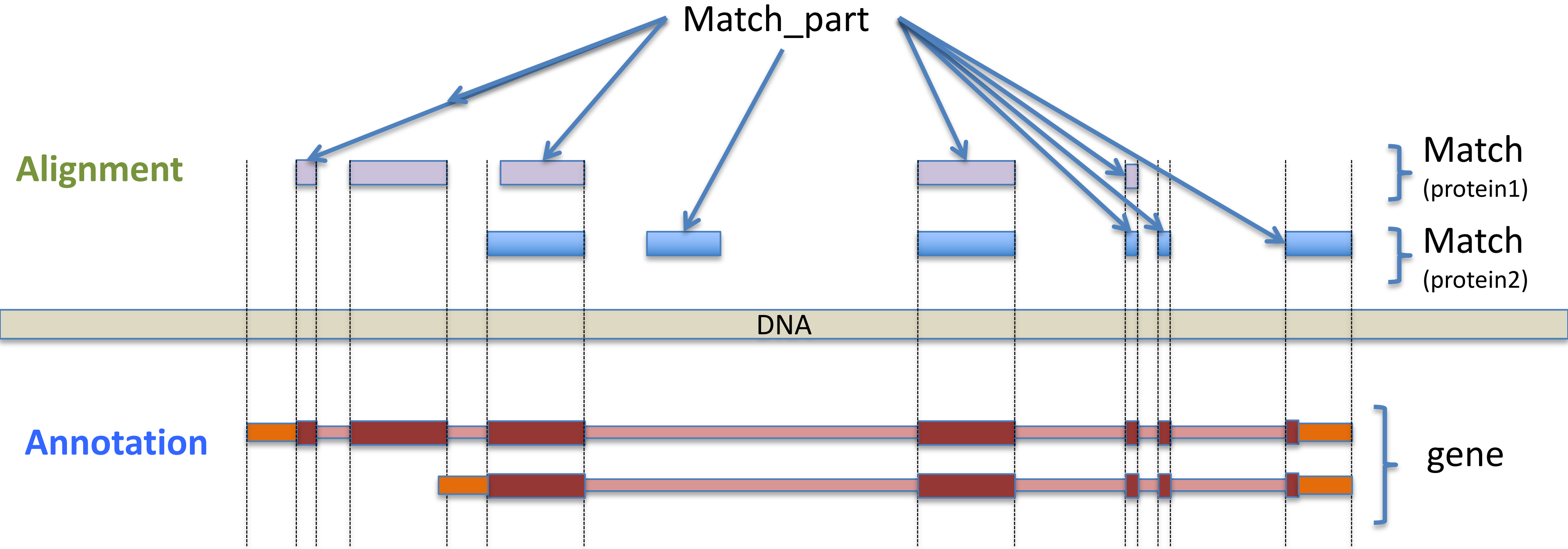
9) attributes
tag=value

! Features are grouped by **parent** relationship

Introduction: Formats: GFF3

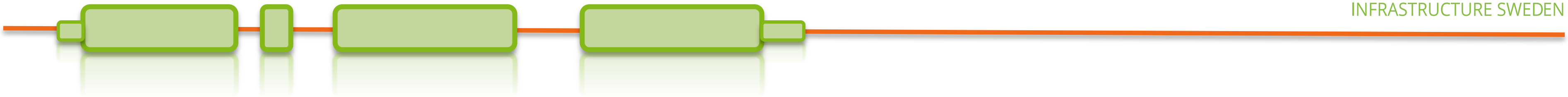


/!\ different type of gff: **annotation** / **alignment** / other

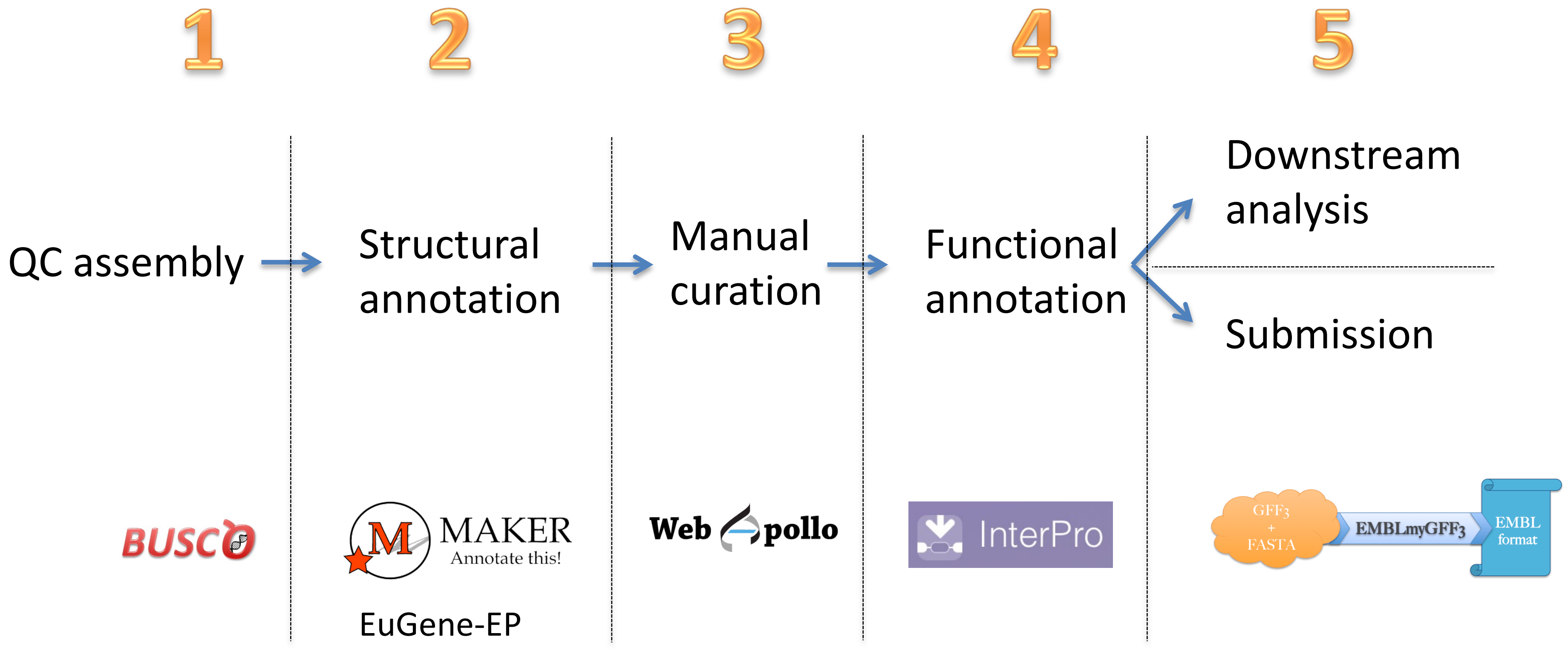


Intron, exon, CDS,splice site, UTR, mRNA, isoforms

Introduction: Overview

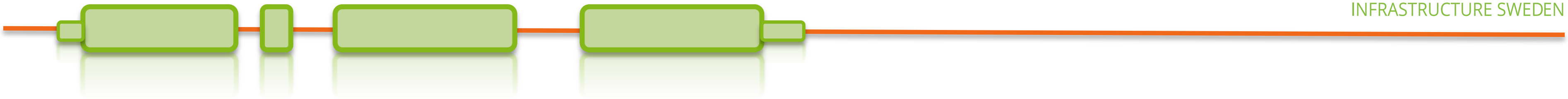


The main steps in genome annotation



Before all annotations

Introduction: Before annotation



- Get the best assembly! The quality of the assembly will heavily influence the quality of the annotation

    - □ SNP-errors can change start/stop-codons
    - □ Indels can cause frame-shifts
    - □ High fragmentation could break loci
    - □ missing loci cannot be annotated

  => Annotation tools have difficulties to deal with those problems

- Freeze the assembly!

  => Updating assembly ~ annotation from scratch

Introduction: Before annotation

Always check :

- Fragmentation (N50, number of sequences, how many small contigs)
- Sanity of the fasta file (Ns, IUPAC, lowercase nucleotides)
- Completeness / duplication / fragmentation BUSCO
- Presence of Organelles
- Other (GC content, how distant from other species)

Introduction: BUSCO

BUSCO used on assembly and annotation

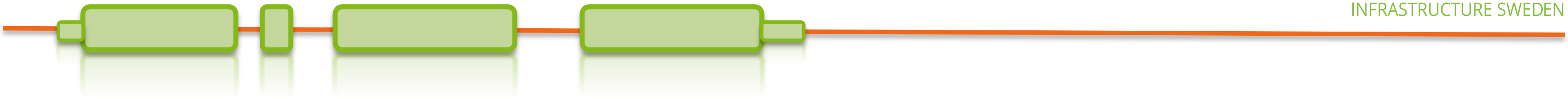
Example of output:

```
# BUSCO version is: 3.0.2
# The lineage dataset is: fungi_odb9 (Creation date: 2016-02-13,
number of species: 85, number of BUSCOs: 290)
#
# Summarized benchmarking in BUSCO annotation for file genome.fa
# BUSCO was run in mode: genome

   C:98.6%[S:97.9%,D:0.7%],F:0.0%,M:1.4%,n:290

   286 Complete BUSCOs (C)
   284 Complete and single-copy BUSCOs (S)
   2   Complete and duplicated BUSCOs (D)
   0   Fragmented BUSCOs (F)
   4   Missing BUSCOs (M)
   290 Total BUSCO groups searched
```

The different approaches



- Similarity-based methods :

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

- *Ab initio* prediction :

  Likelihood based methods

- Hybrid 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

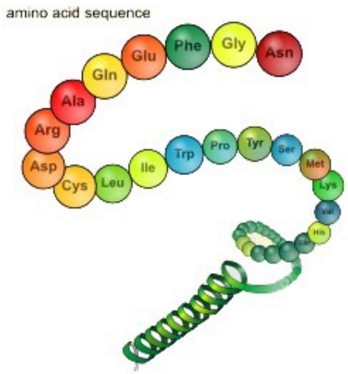
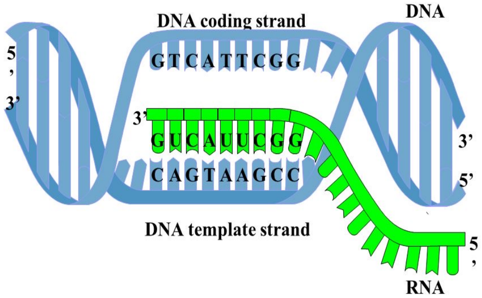
- Chooser, combiner approaches :

  These combine gene predictions of other gene finders

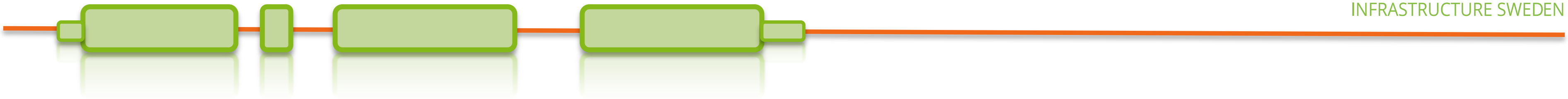
- Pipelines :

  These combine multiple approaches

Types data used vs methods

Annotation approach	∅	Proteins	Transcripts
	This space intentionally left blank.	Known amino acid sequences from other organisms 	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

Intrinsic / *ab initio*



Strengths :

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

Limits :

- No UTR
- No alternatively spliced transcripts (augustus does)

- 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

Exercises

https://nbisweden.github.io/workshop-genome_annotation_elixir/labs/augustus

The different approaches

- Similarity-based methods :

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

- *Ab initio* prediction :

  Likelihood based methods

- Hybrid 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

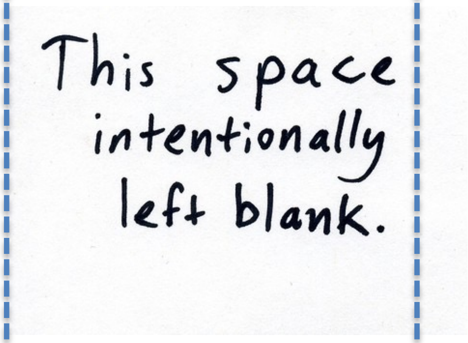
- Pipelines :

  These combine multiple approaches

Types data used vs methods

Annotation approach

Ø



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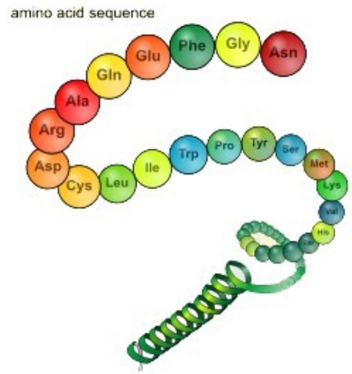
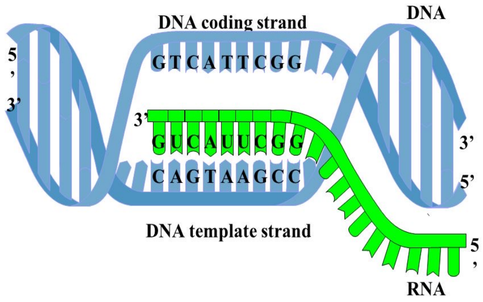
Proteins

Known amino acid sequences from other organisms



Transcripts

Assembled from RNA-seq or downloaded ESTs



Annotation approach	Ø	Proteins	Transcripts
Similarity		X	X
Pure ab initio	X		
Hybrid	X	X	X
Comparative	X	X	X
Chooser/combiner	X	X	X
Pipeline	X	X	X

Get the data

NATIONAL BIOINFORMATICS INFRASTRUCTURE SWEDEN

- Genome
  - Fasta or gff format
- Repeats
  - Fasta or gff format
- Proteins
  - Fasta format
  - Uniprot/swissprot
  - Close related species
- RNAseq
  - Fasta or gff format
  - Same individual best
  - SRA (Sequence Read Archive)

First of all: Repeat Masking

- Repeatmodeler to find new repeats
  - http://www.repeatmasker.org/RepeatModeler/

- Repeatmasker to mask known repeats
  - http://www.repeatmasker.org

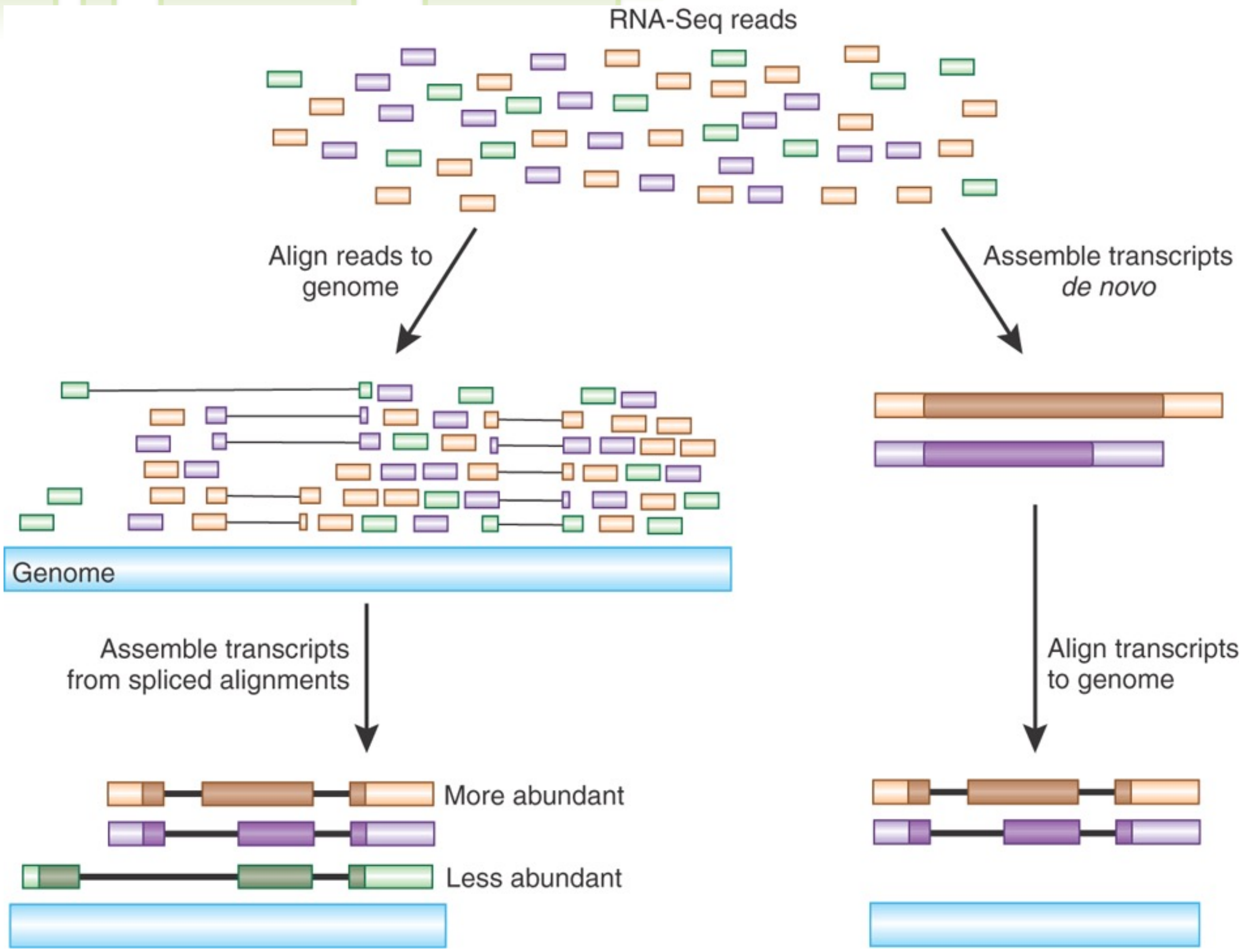
+ Save time

+ Increase quality of the gene coding annotation

Extrinsic data

- Proteins :
  - Related to pre-existing data
  - Proteins from model organisms often used => bias?
  - Proteins can be incomplete
  - Protein can be wrong (PE)
  - No UTR
- RNAseq :
  - Hard to catch low expressed / peculiar expressed (stage of life, condition, etc…) / isoforms
  - short-reads:
    - Transcriptome assembly errors
  - Long-reads:
    - error rate / frameshift / indels

Assembly of transcripts


RNA-Seq reads
Align reads to genome
Assemble transcripts *de novo*
Genome
Assemble transcripts from spliced alignments
Align transcripts to genome
More abundant
Less abundant

Haas and Zody, Nature Biotechnology 28, 421–423 (2010)

De-novo transcriptome assembly

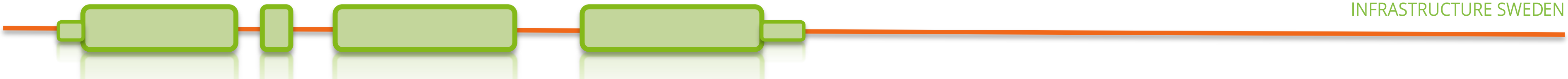
- Most used programs (latest release date):
  - Trinity (March 2021)
  - SOAPdenovo-Trans (Aug 2017)
  - Trans-ABySS (Feb 2018)
  - Velvet+Oases (March 2015)
- Originally SOAPdenovo, ABySS and Velvet for de novo genome assembly
- "SOAPdenovo-Trans incorporates the error-removal model from Trinity and the robust heuristic graph traversal method from Oases."

Trinity, Grabherr et al. 2011

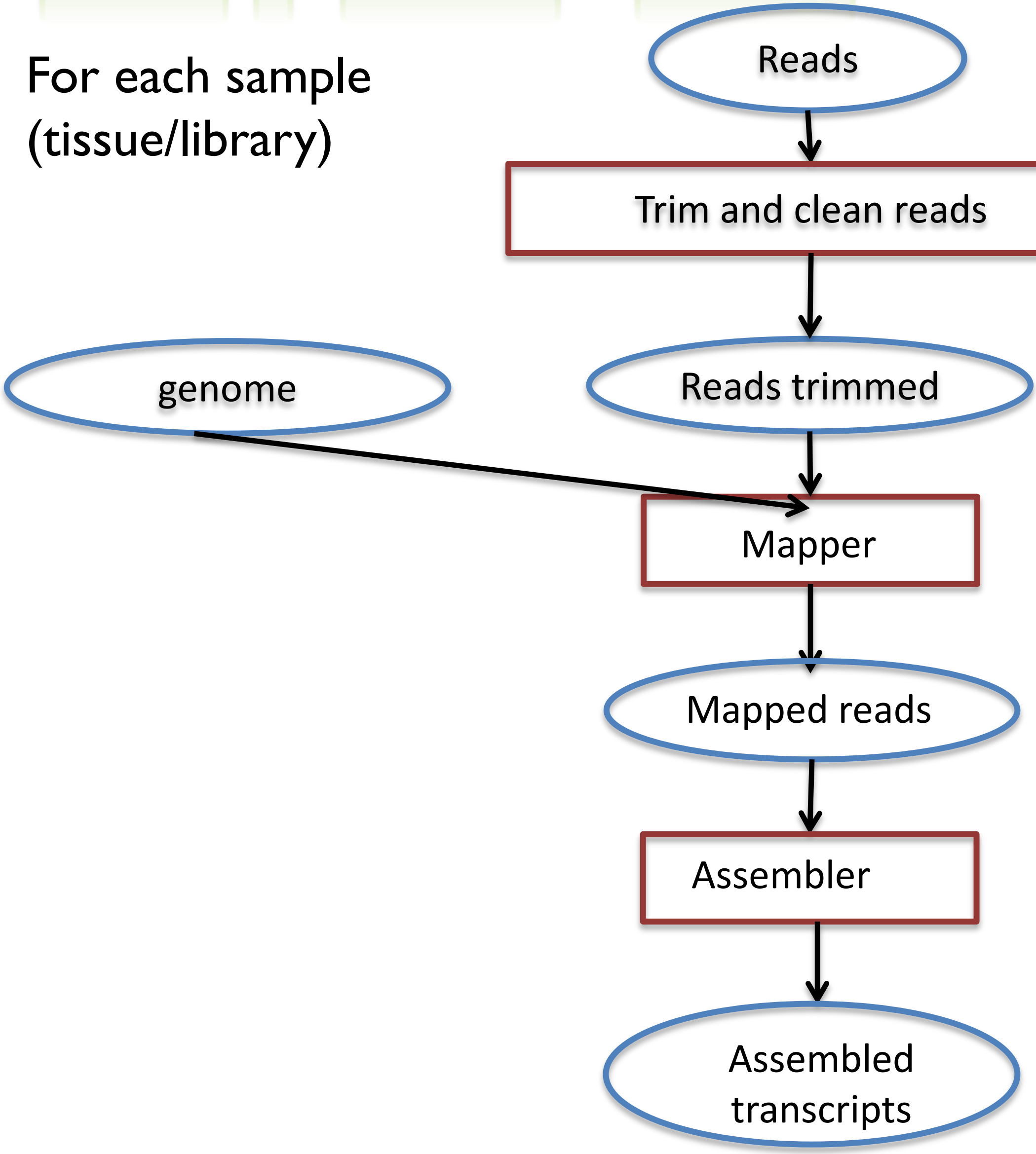
De-novo transcriptome assembly

- No reference needed
- Many programs available
- Lots of potential transcripts. Filter!

Genome guided transcriptome assembly



For each sample (tissue/library)



E.g : trimmomatic, fastp

E.g : hisat2, star

E.g : stringtie, cufflinks

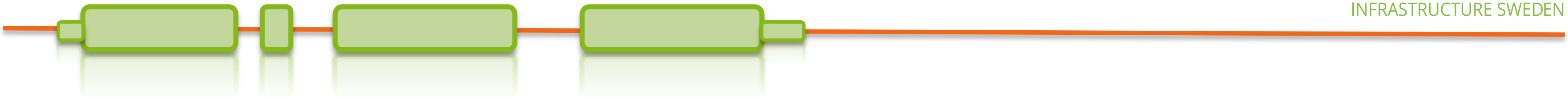
Genome-guided transcriptome assembly

- Need a very good reference (genome most of the time)
- Can use existing annotation (GTF/GFF file) (in option for stringtie)
- Can detect novel transcripts

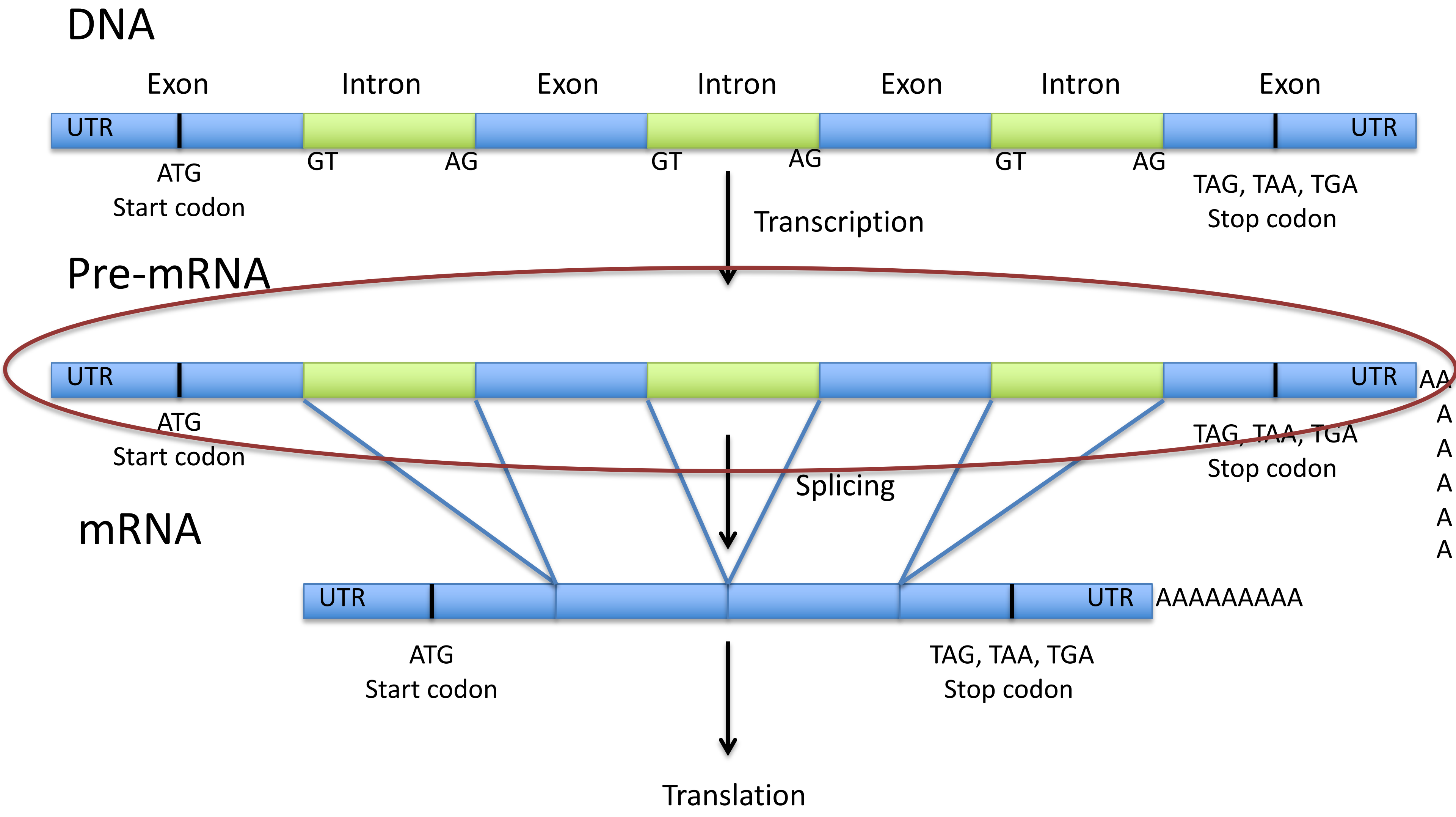
https://github.com/NBISweden/pipelines-nextflow/tree/master/TranscriptAssembly

RNAseq

How does it look
when it does not look good?



Types of data used: RNA-seq



DNA

Exon Intron Exon Intron Exon Intron Exon

UTR UTR

ATG
Start codon

GT AG GT AG GT AG

TAG, TAA, TGA
Stop codon

Transcription

Pre-mRNA

UTR UTR AA
A
A
A
A
A

ATG
Start codon

TAG, TAA, TGA
Stop codon

Splicing

mRNA

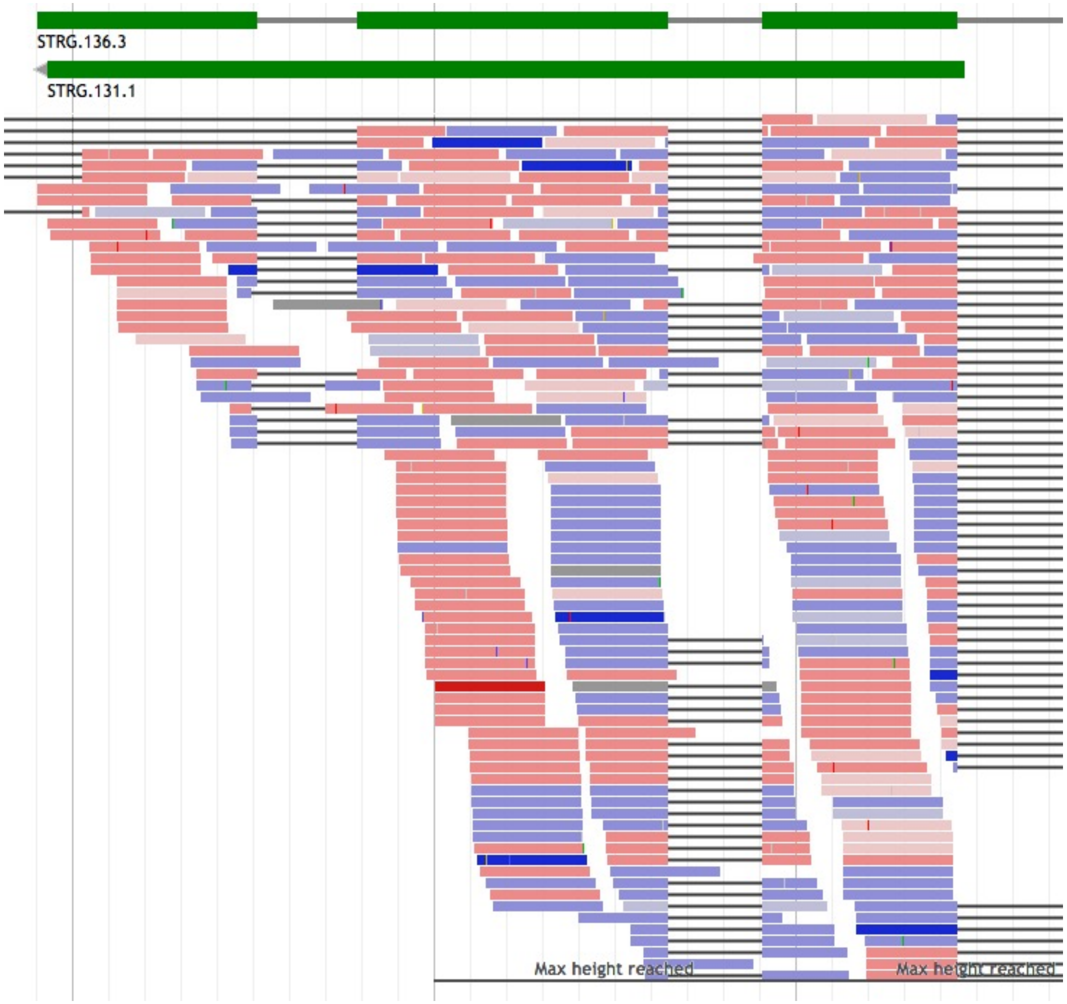
UTR UTR AAAAAAAAA

ATG
Start codon

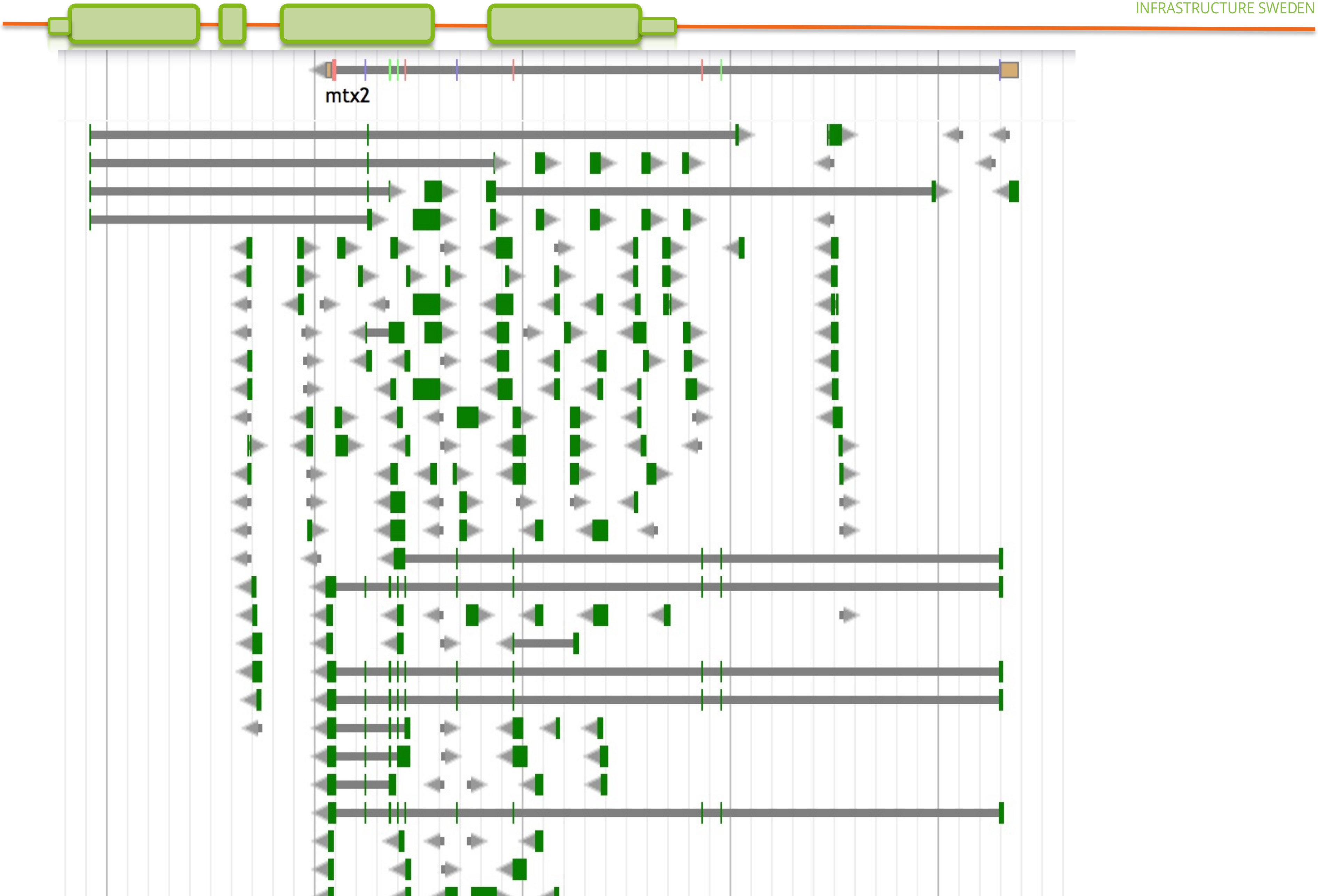
TAG, TAA, TGA
Stop codon

Translation

RNA-seq – pre-mRNA noise



Trinity noise



RNAseq conclusion

- RNAseq data should always be included in an annotation project
- From the same organism as the genomic data => unbiased
- Can be used before annotation or after to improve an annotation already existing
- Sample different tissues or life stages if possible
- Avoid gonads and brain; muscle is good

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

- Combining method is best if possible

MAKER lecture

https://nbisweden.github.io/workshop-genome_annotation_elixir/lectures/Structural_annotation_MAKER_Norway2021.pptx

Exercises

https://nbisweden.github.io/workshop-genome_annotation_elixir/labs/maker_evidence

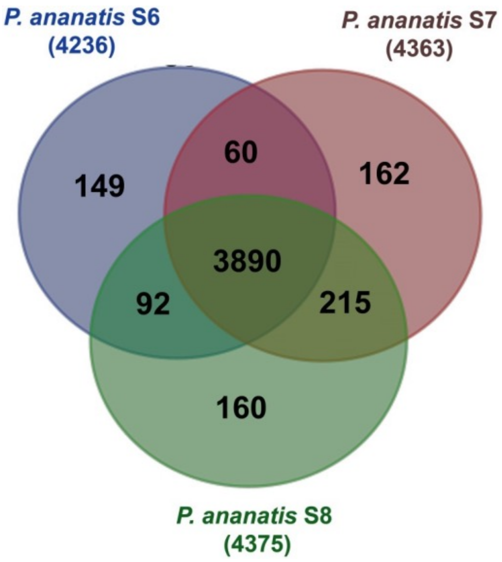
https://nbisweden.github.io/workshop-genome_annotation_elixir/labs/augustus_training

https://nbisweden.github.io/workshop-genome_annotation_elixir/labs/maker_abinitio_evidence_driven

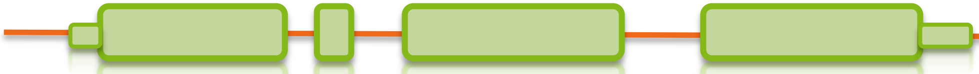
After structural annotation
Assessing an annotation

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



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

Exercises

https://nbisweden.github.io/workshop-genome_annotation_elixir/labs/annotation_assessment

Closing remarks

Closing remarks

- >100 annotation tools – as many methods

(https://github.com/NBISweden/GAAS/blob/master/annotation/knowledge/annotation_tools_genome.md)

- 6 main class of approaches (Similarity-based, *ab initio*, hybrid, comparative, combiner, pipeline)

How to choose Method:

- Scientific question behind (need of a <u>conservative</u> annotation vs <u>exhaustive</u>)

- 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

- 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 !!

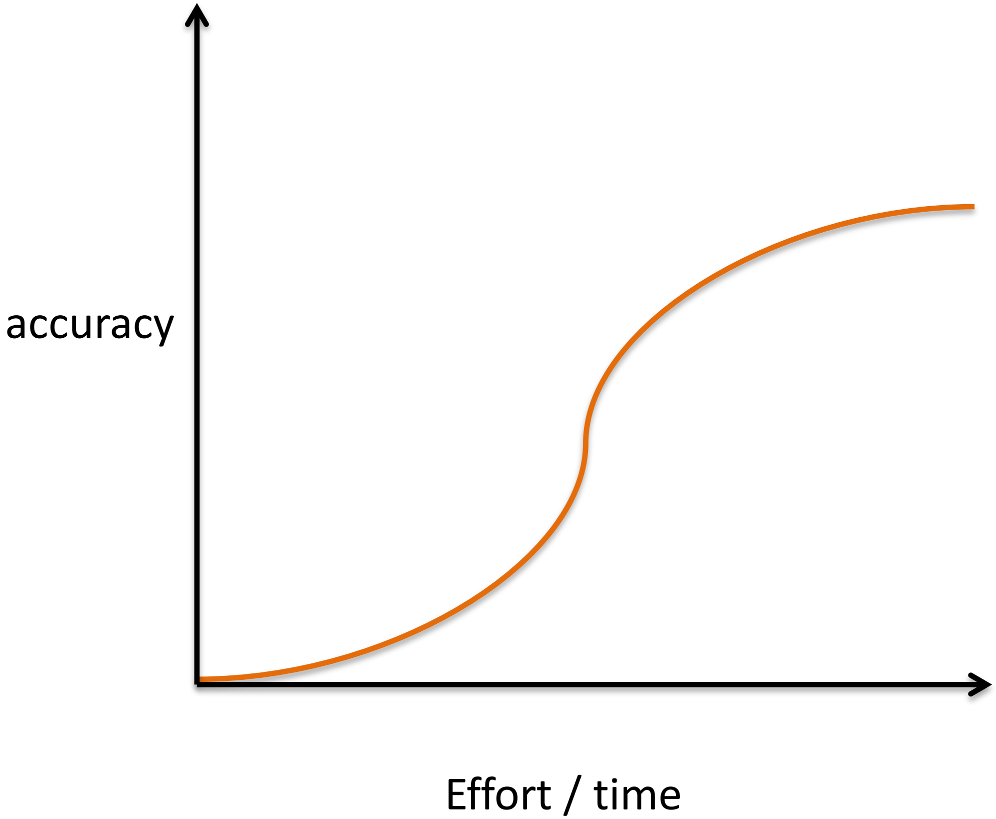
- Annotation requires **manual curation**

- 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

Closing remarks

Effort versus accuracy



THE END

https://github.com/NBISweden/GAAS
https://github.com/NBISweden/AGAT
https://github.com/NBISweden/pipelines-nextflow