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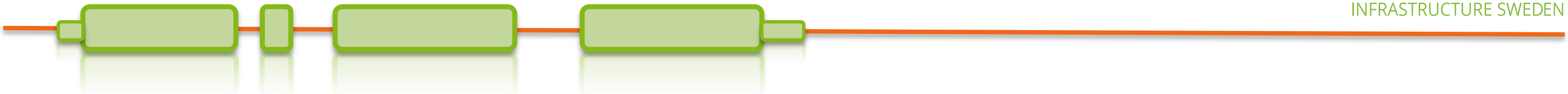
Jacques Dainat, PhD

How to do a genome annotation?

Tromsø – May 2021

Introduction: Formats



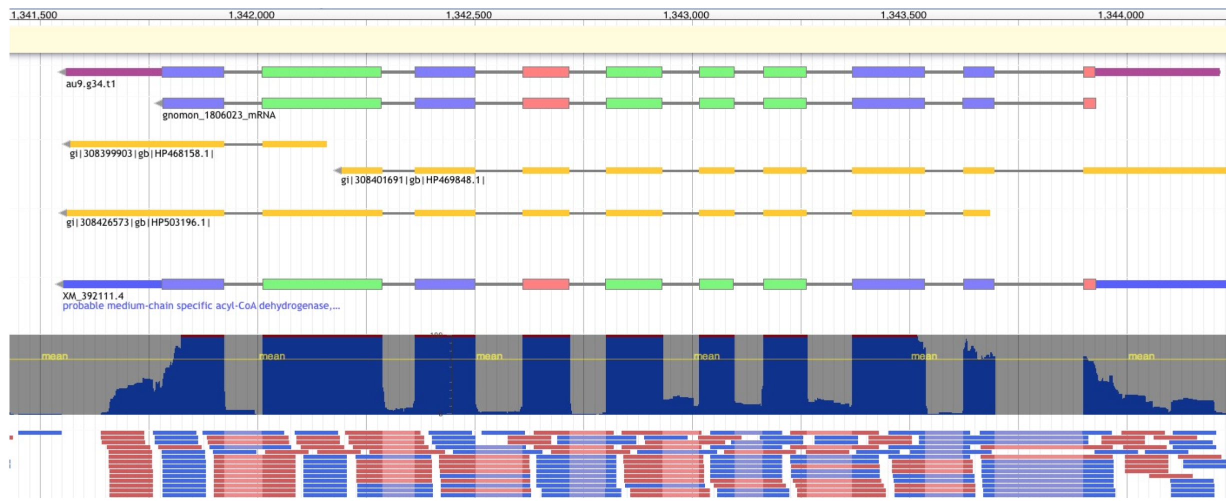
From a genome…

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



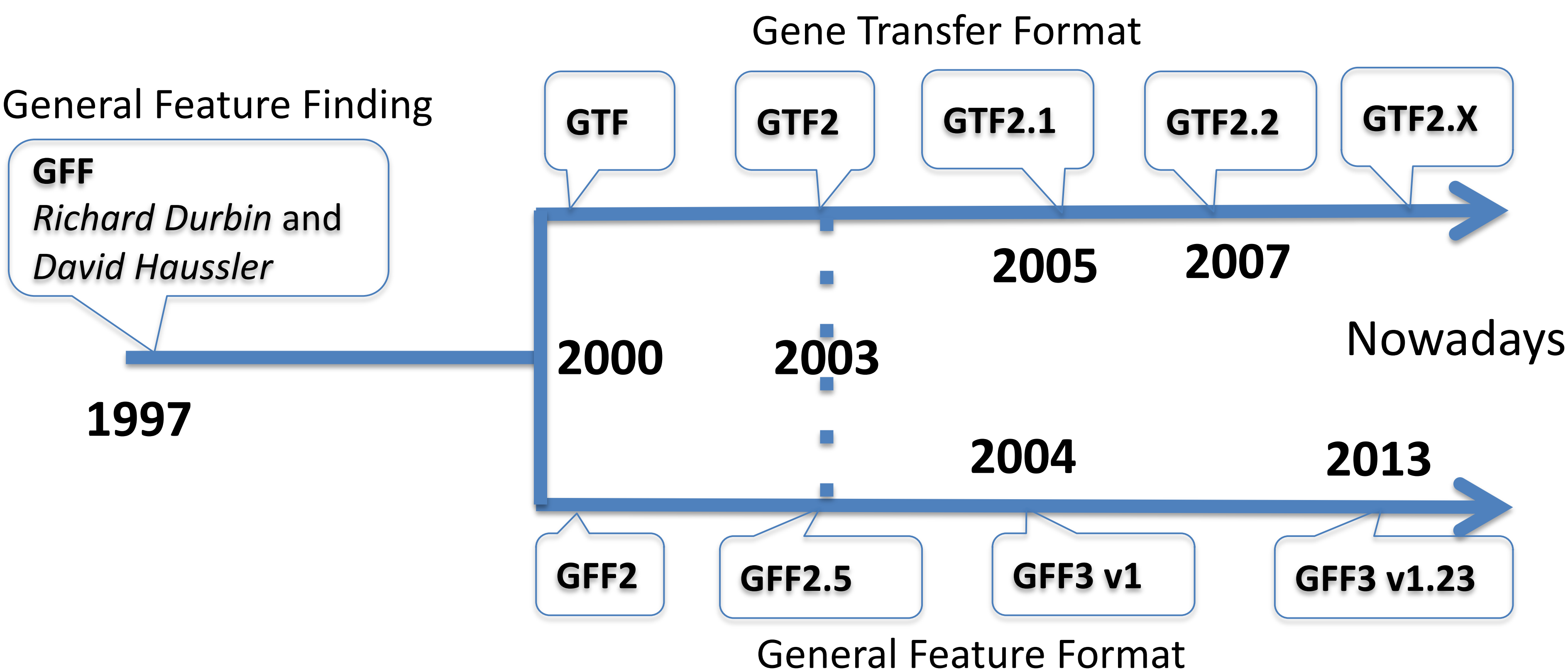
- 9 columns
- 1 feature = 1 line

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



Introduction: Formats: GTF2.X

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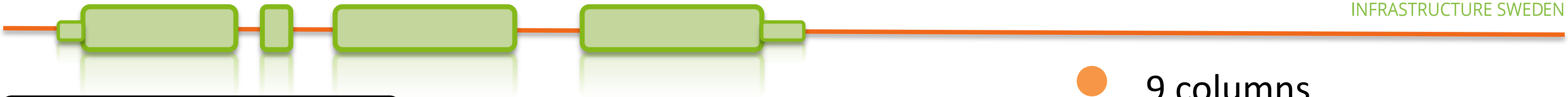


- 9 columns
- 1 feature = 1 line

```
#!genome-build GRCz11
#!genome-date 2017-05
```

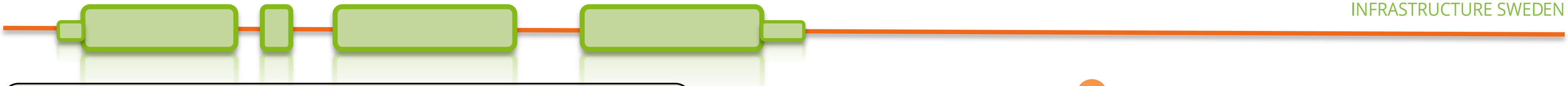
Header

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

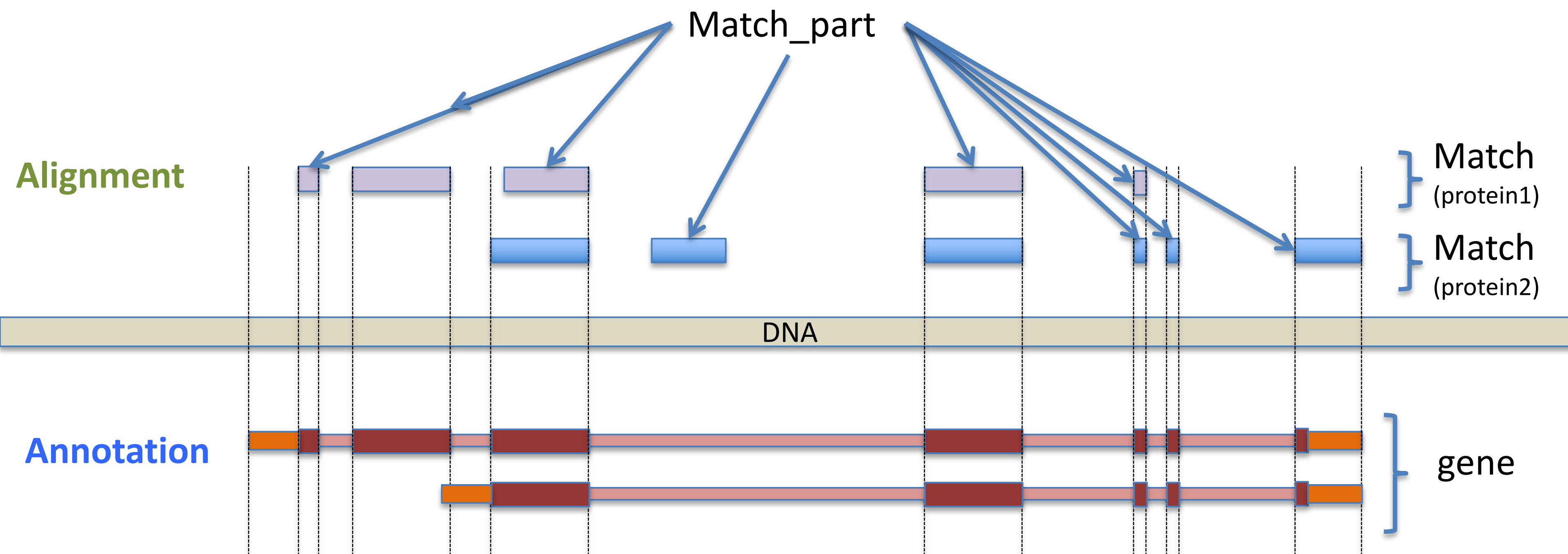
- 9 columns
- 1 feature = 1 line

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*
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

! 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

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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!	WebApollo	InterPro	
	EuGene-EP			

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

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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

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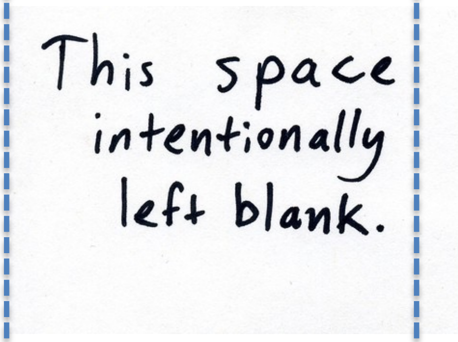
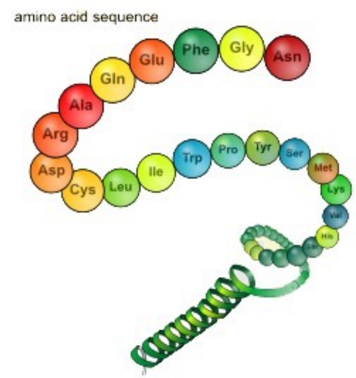
Types data used vs methods

Ø



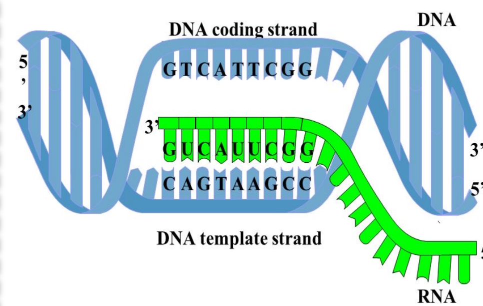
Proteins

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

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 :

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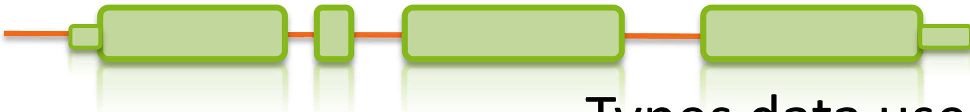
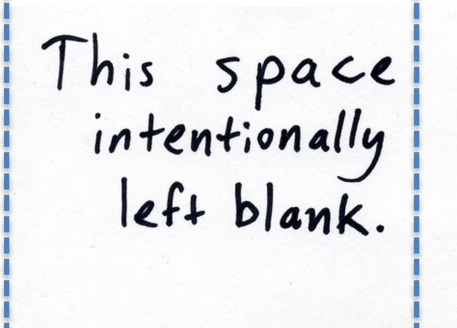
The different approaches



Types data used vs methods

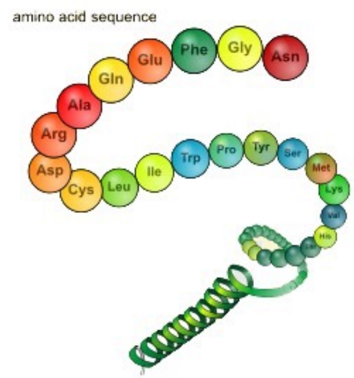
Annotation approach

Ø



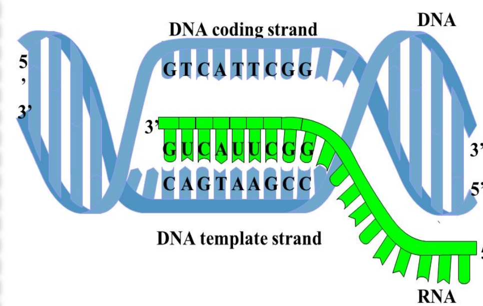
Proteins

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

- 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

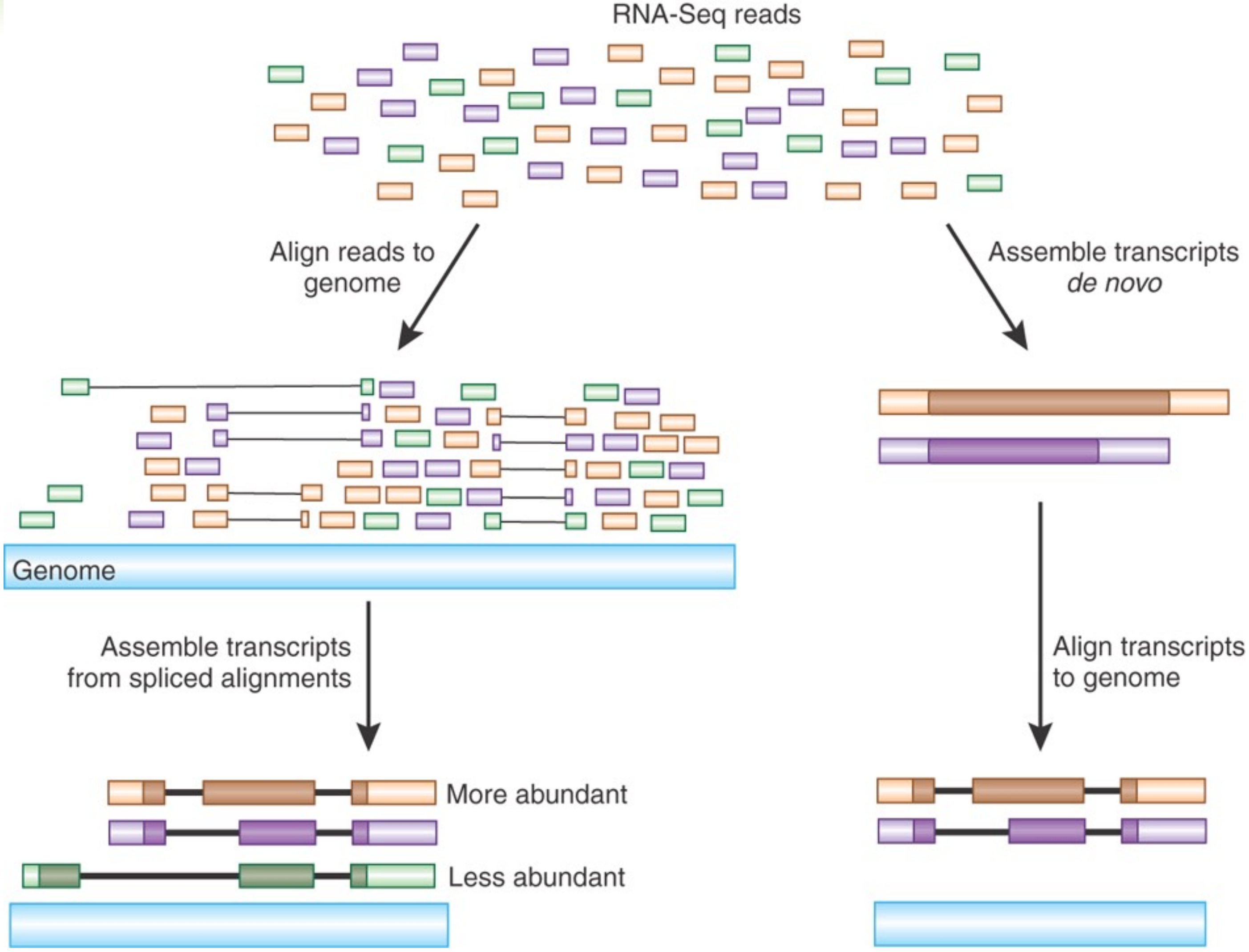
+ Increase quality of the gene coding annotation

http://weatherby.genetics.utah.edu/MAKER/wiki/index.php/Repeat_Library_Construction--Basic
https://www.biostars.org/p/411101/
http://weatherby.genetics.utah.edu/MAKER/wiki/index.php/Repeat_Library_Construction-Advanced

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



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.”

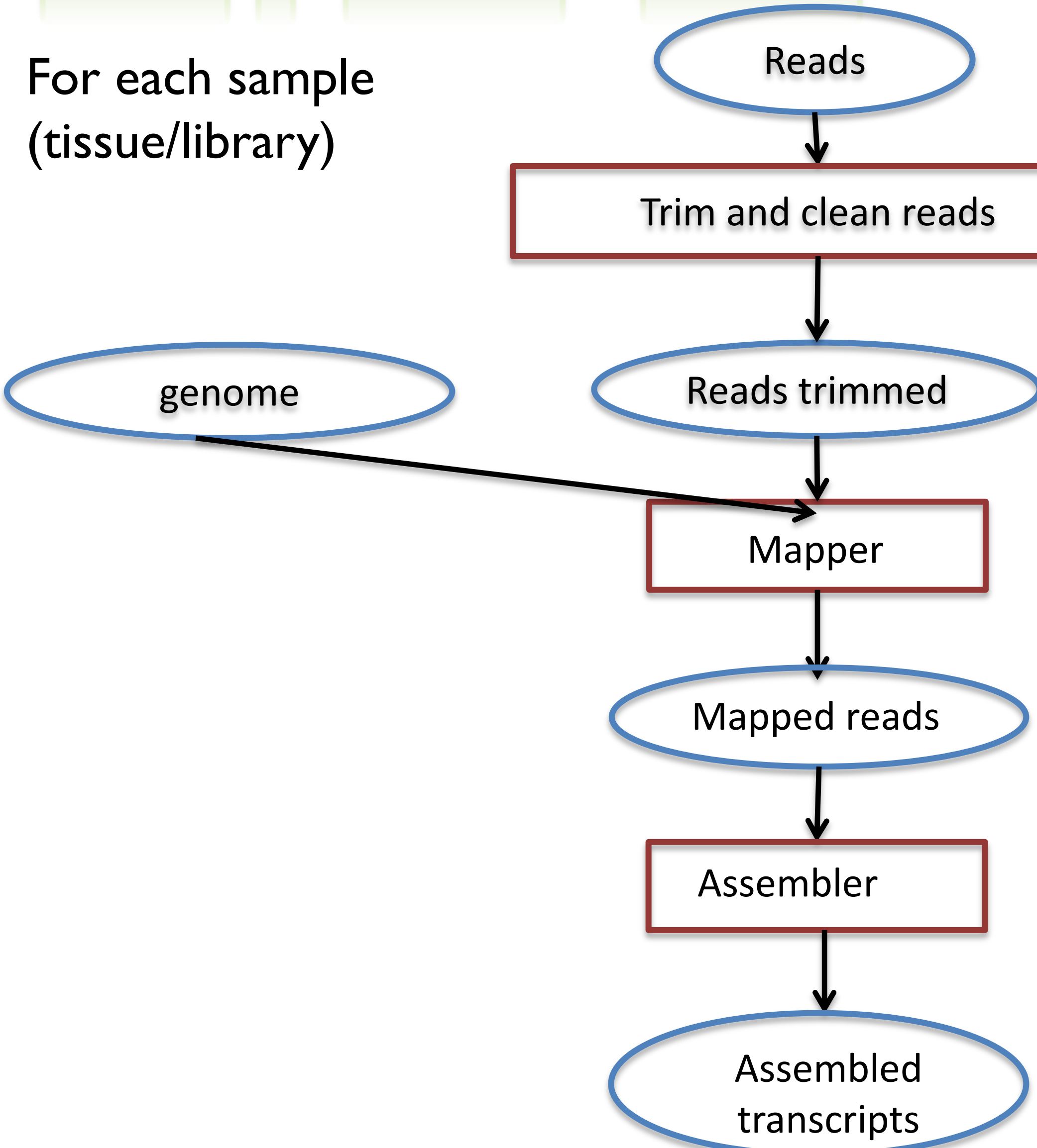
Trinity, Grabherr et al. 2011

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

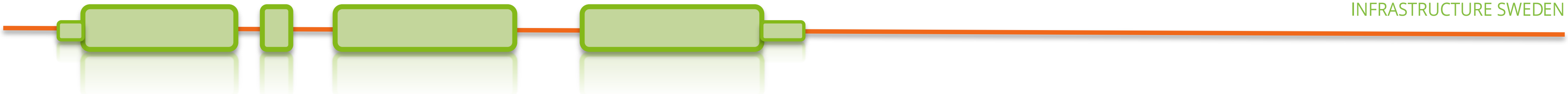
Genome-guided transcriptome assembly

- 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

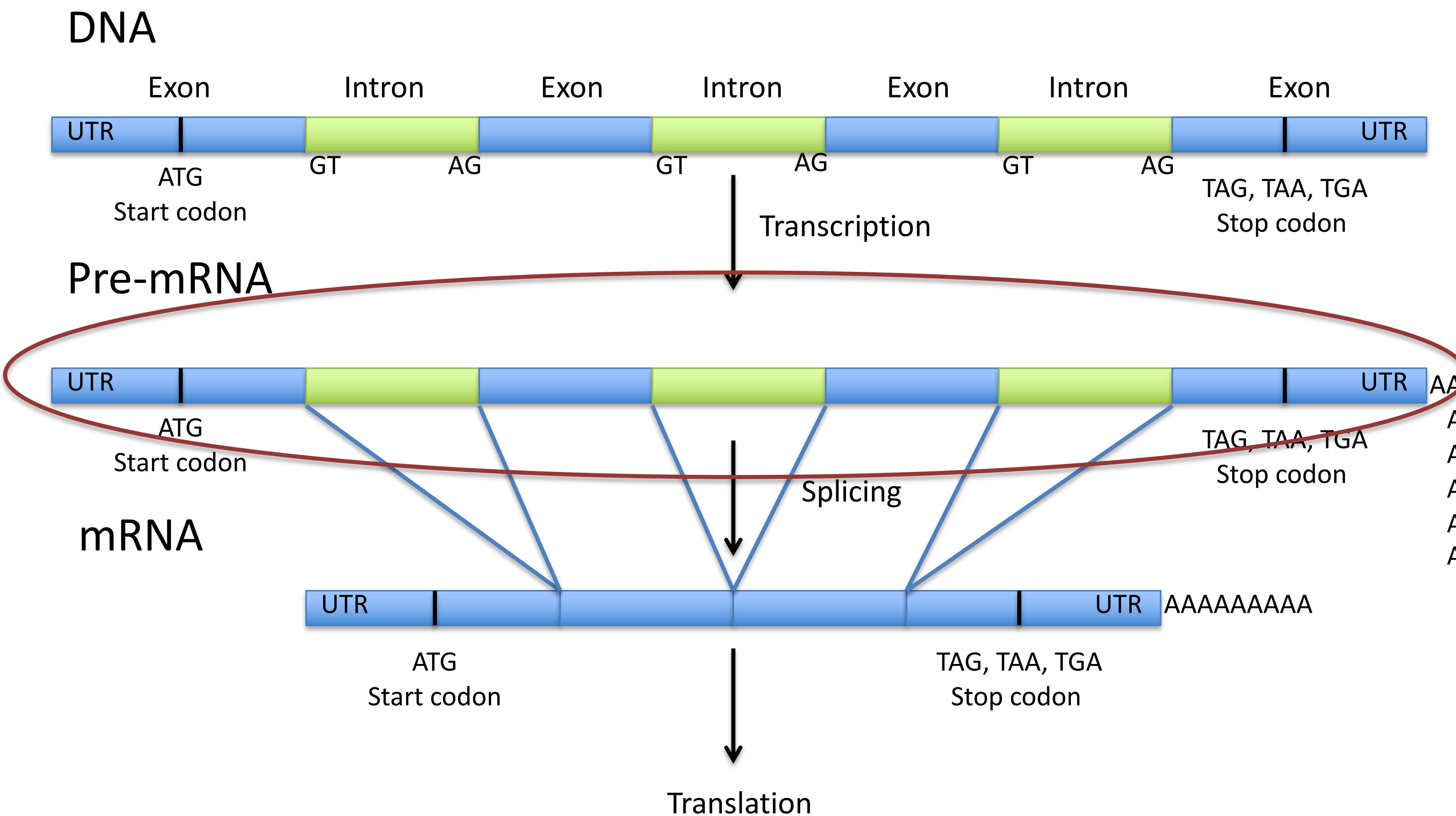
https://github.com/NBISweden/pipelines-nextflow/tree/master/TranscriptAssembly
https://nbisweden.github.io/workshop-genome_annotation/labs/rnaseq_guided_assembly

RNAseq

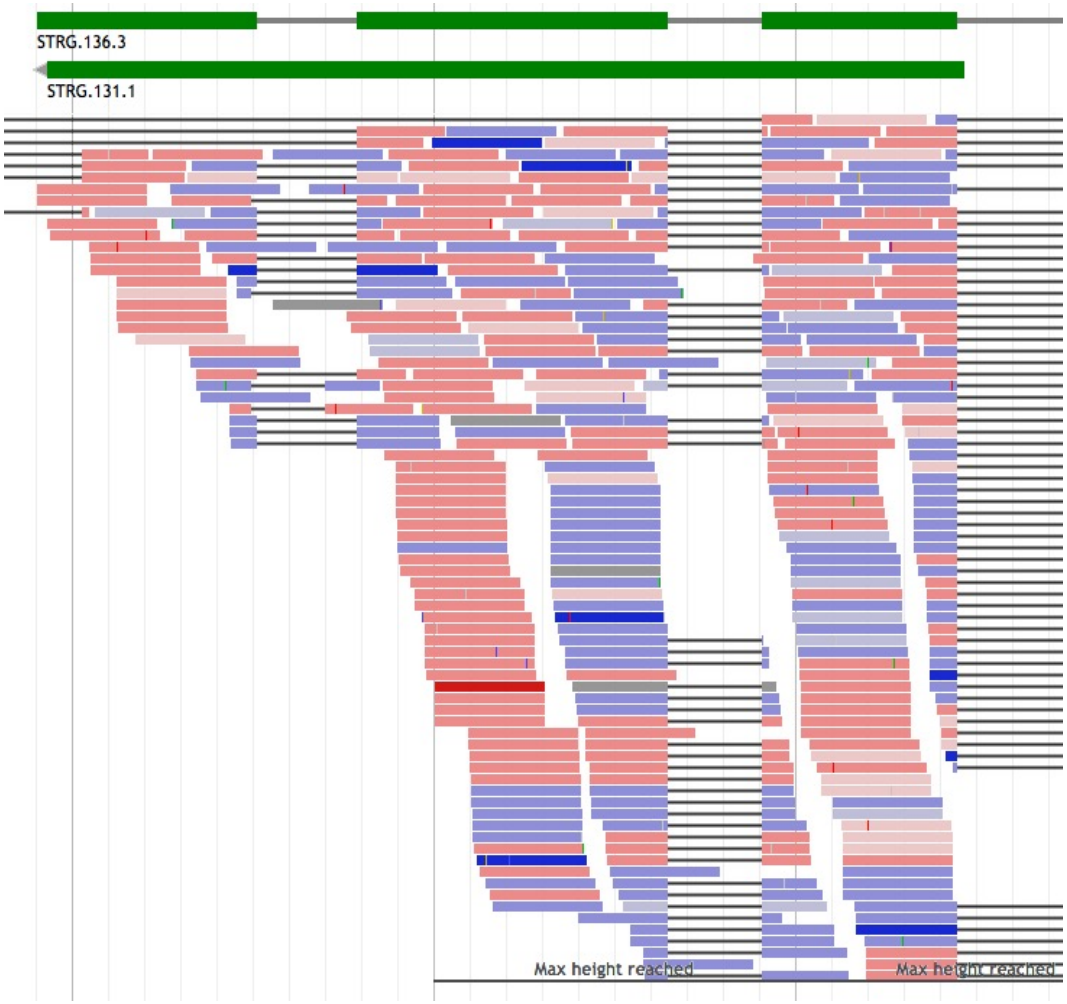
How does it look
when it does not look good?



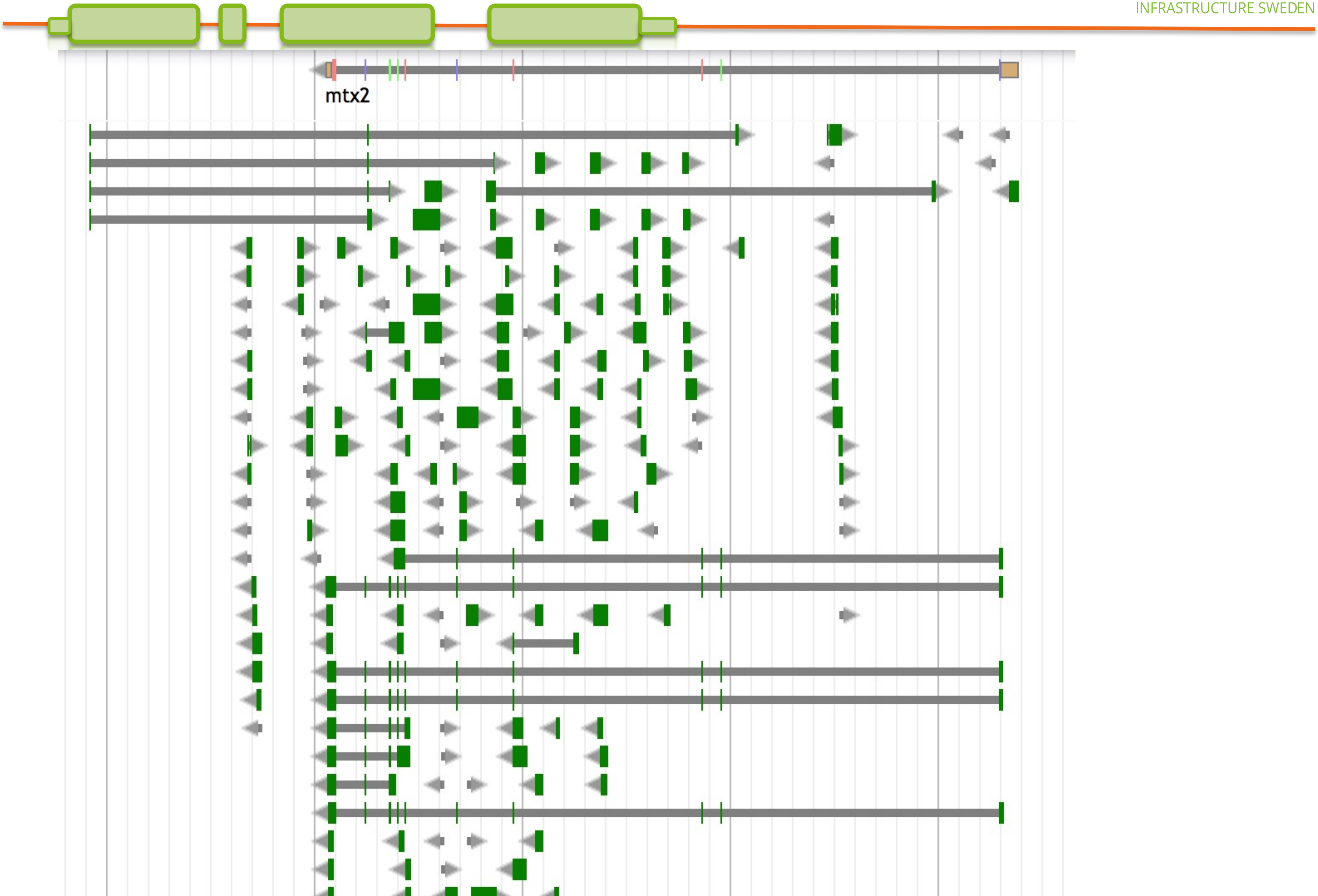
Types of data used: RNA-seq



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

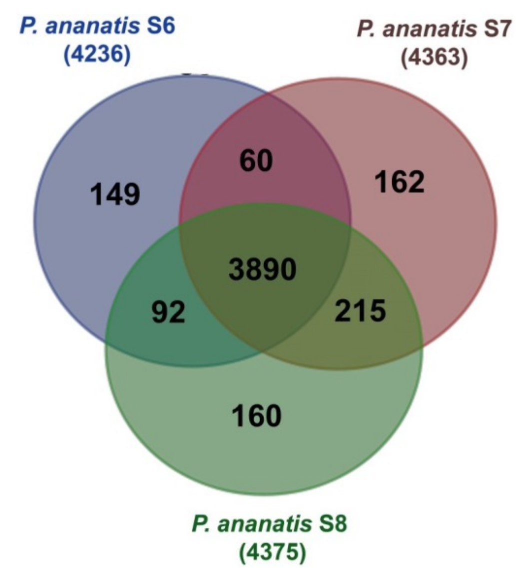
Exercises

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 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

- 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 !!

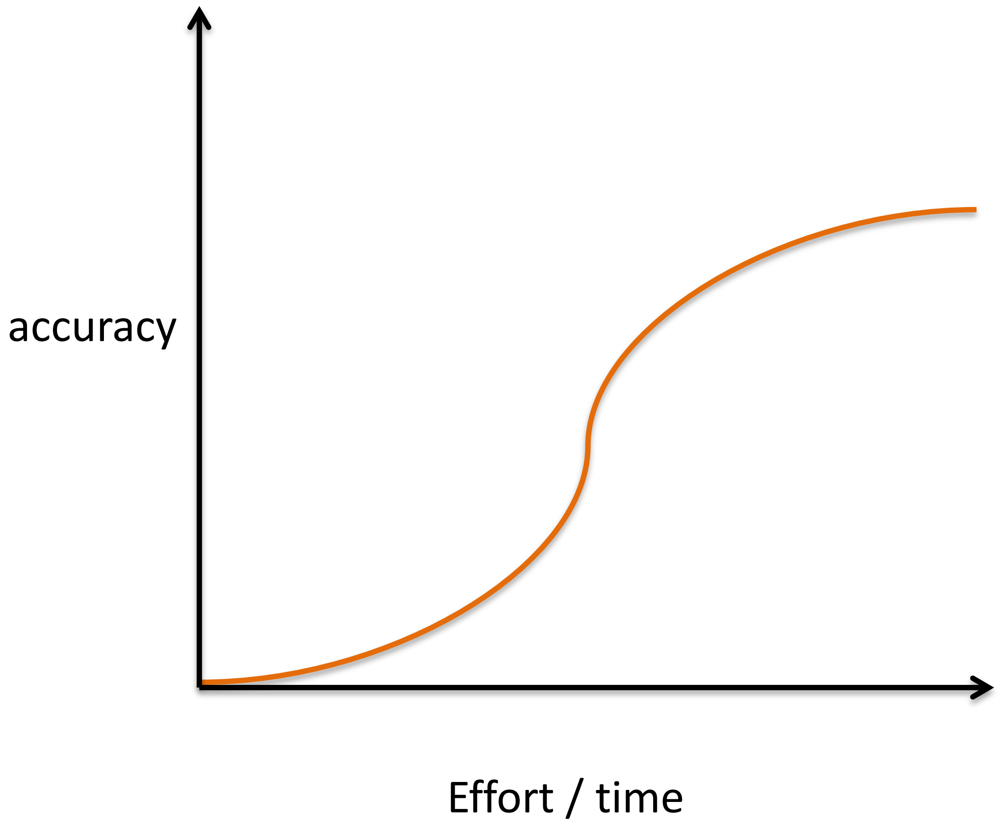
- Annotation requires **manual curation**

- 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