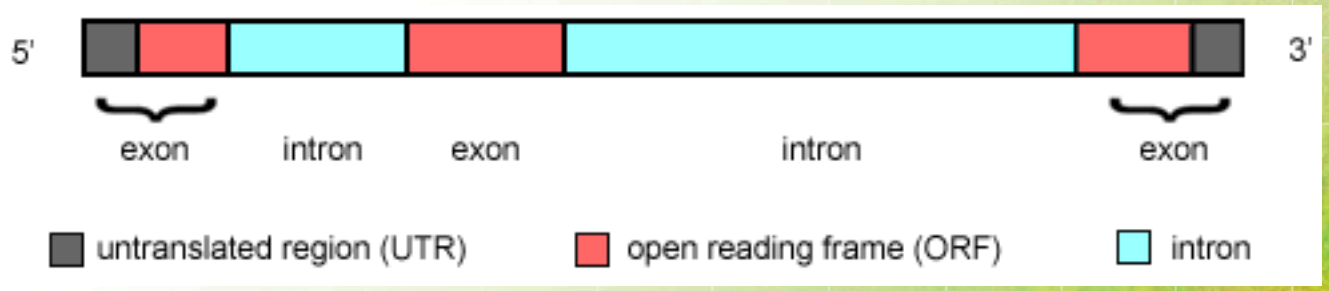
Introduction to genome annotation - practical information



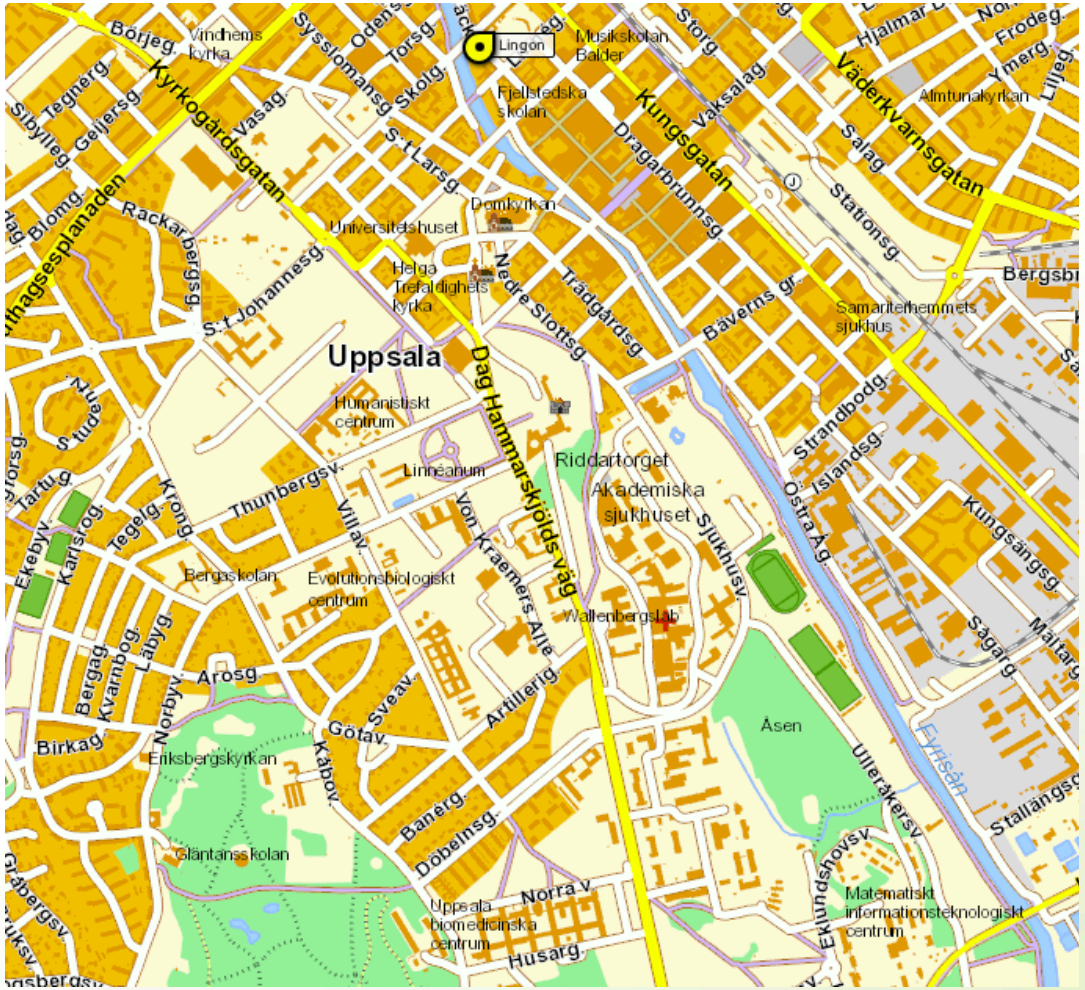
Enabler for Life Sciences

Practical info

- Coffee breaks
- Lunch
- Dinner at

  Lingon 18.00

  Svartbäcksg. 30



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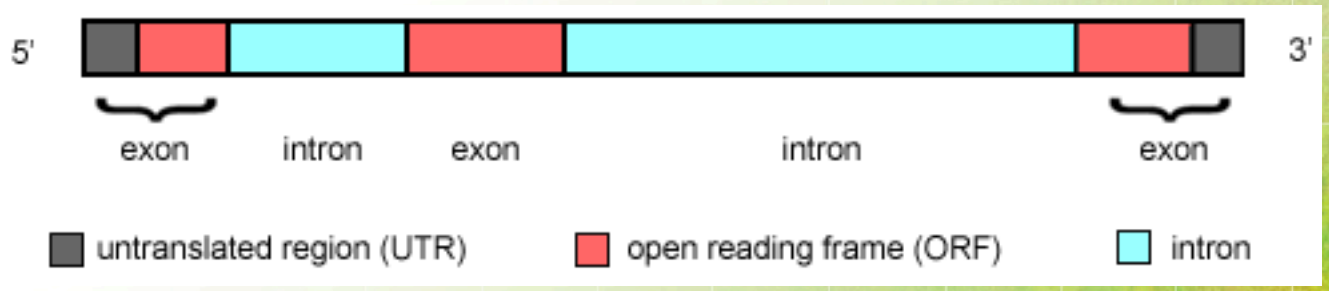
BILS Bioinformatics Infrastructure for Life Sciences

SciLifeLab

Understanding annotation



Henrik Lantz, BILS/SciLifeLab

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Lecture synopsis

- What is annotation?
- Structural genome annotation
- Types of data used
- Transcriptome annotation
- Functional annotation

What is annotation?

- Identification of regions of interest in sequence data

From a genome…

```
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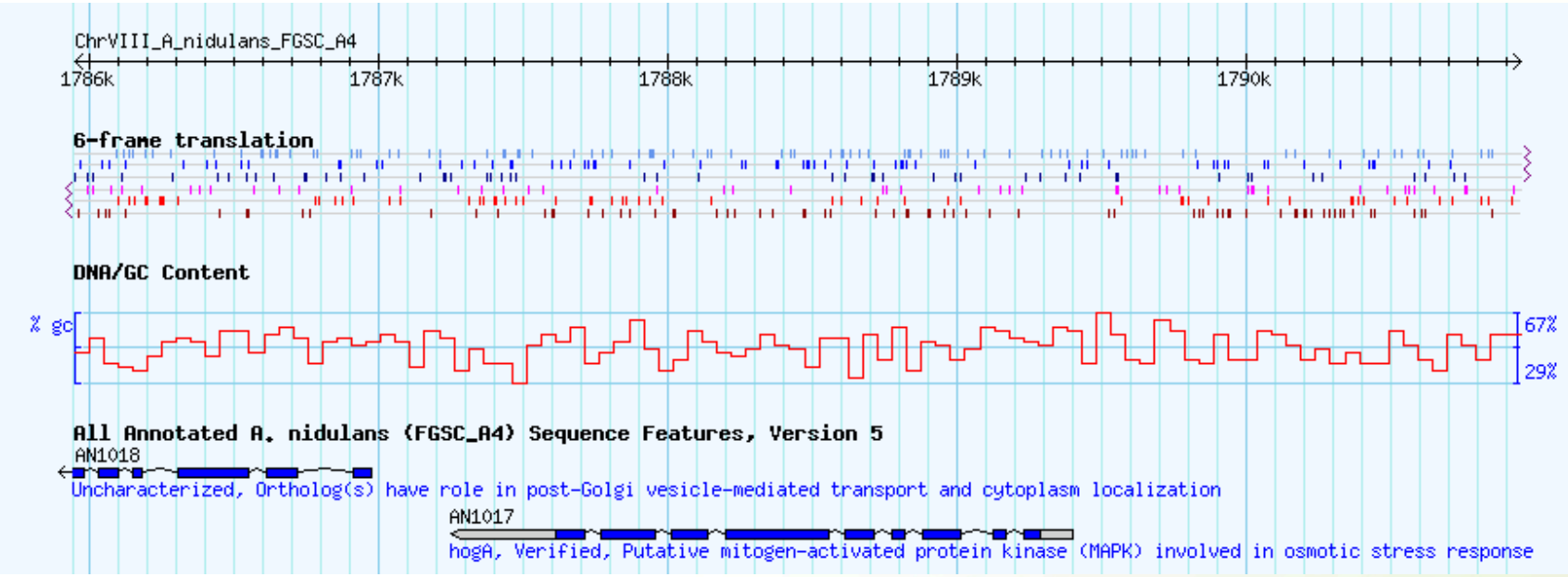
…to an annotated gene



GFF file format

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scaffold_7	maker	exon	86666	86750	.	+	.	ID=CFF5DDA190832937C45A0D2E674C9C26:exon:19;Parent=CFF5DDA190832937C45A0D2E674C9C26
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scaffold_7	maker	CDS	110231	110356	.	+	2	ID=CFF5DDA190832937C45A0D2E674C9C26:cds;Parent=CFF5DDA190832937C45A0D2E674C9C26
scaffold_7	maker	CDS	111579	111669	.	+	2	ID=CFF5DDA190832937C45A0D2E674C9C26:cds;Parent=CFF5DDA190832937C45A0D2E674C9C26
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maker.gff
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GFF3 file format

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GTF file format

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BILS Bioinformatics Infrastructure for Life Sciences

SciLifeLab

GTF file format

Seqid	source	type	start	end	score	strand	phase	attributes
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Why is annotation important?

Example: Differential expression

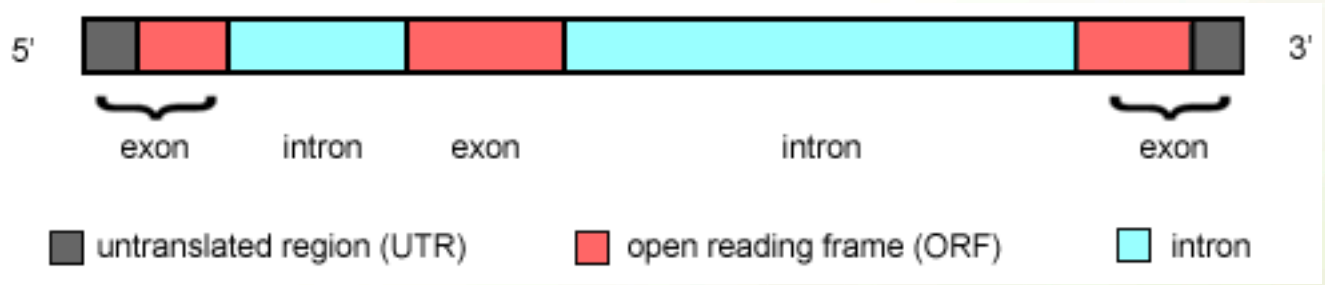
Why is annotation important?

RNA-seq reads

Genome

There are two major parts of annotation

- 1) Structural: Find out where the regions of interest (usually genes) are in the genome and what they look like. How many exons/introns? UTRs? Isoforms?

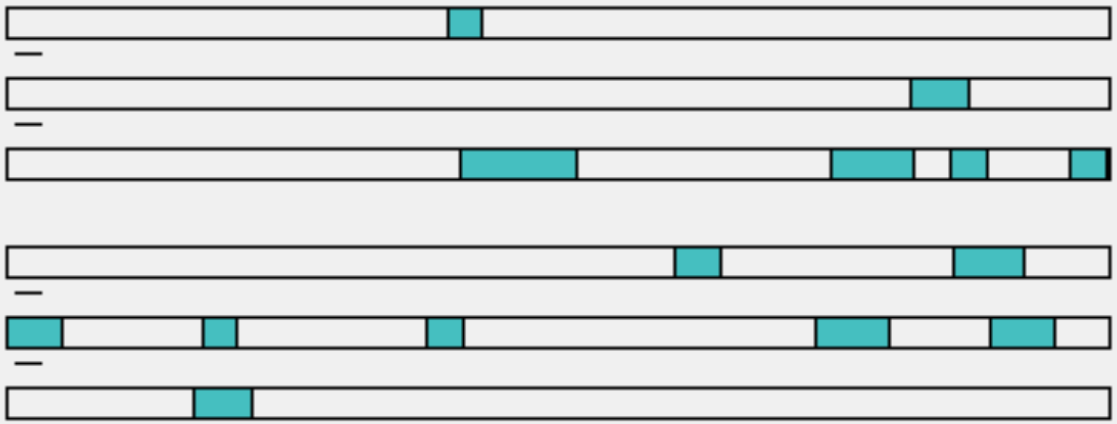


- 2) Functional: Find out what the regions do. What do they code for?

Open reading frames

Anonymous

View | 1 GenBank | Redraw | 100 | SixFrames



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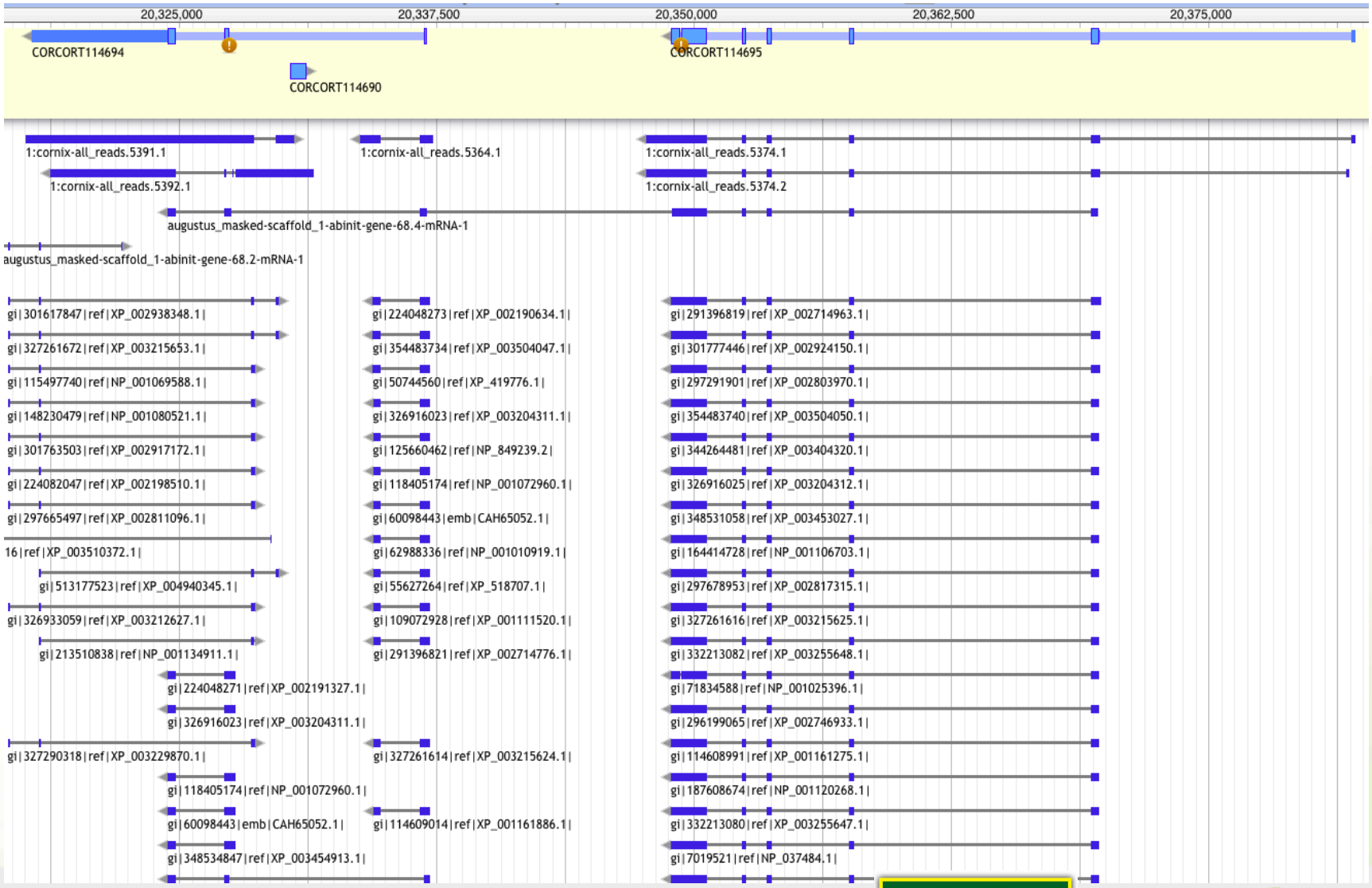
+3	1383	1736	354
+3	2511	2756	246
-2	2465	2686	222
-1	2880	3092	213
-2	2996	3187	192
-3	574	753	180
+2	2753	2929	177
-2	2	172	171
-1	2034	2171	138
+3	3237	3349	114
+3	2874	2984	111
-2	1283	1393	111
+1	1345	1446	102
-2	599	700	102

Difficult in practice



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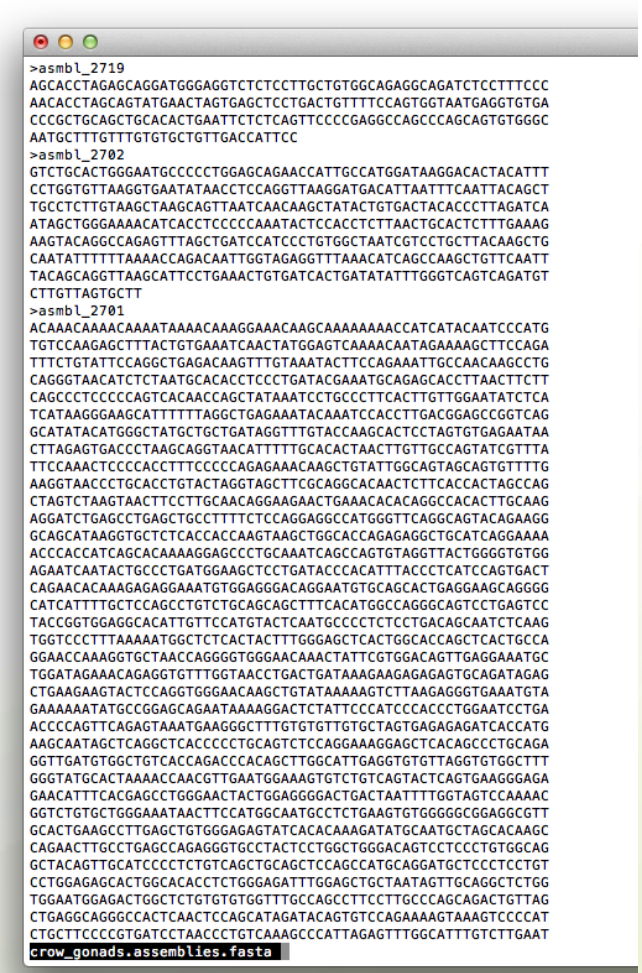
Combine data - use Maker!

- External data - proteins, rna-seq (incl. ESTs)
- Ab-initio gene finders
- (Lift-overs from closely related genomes)

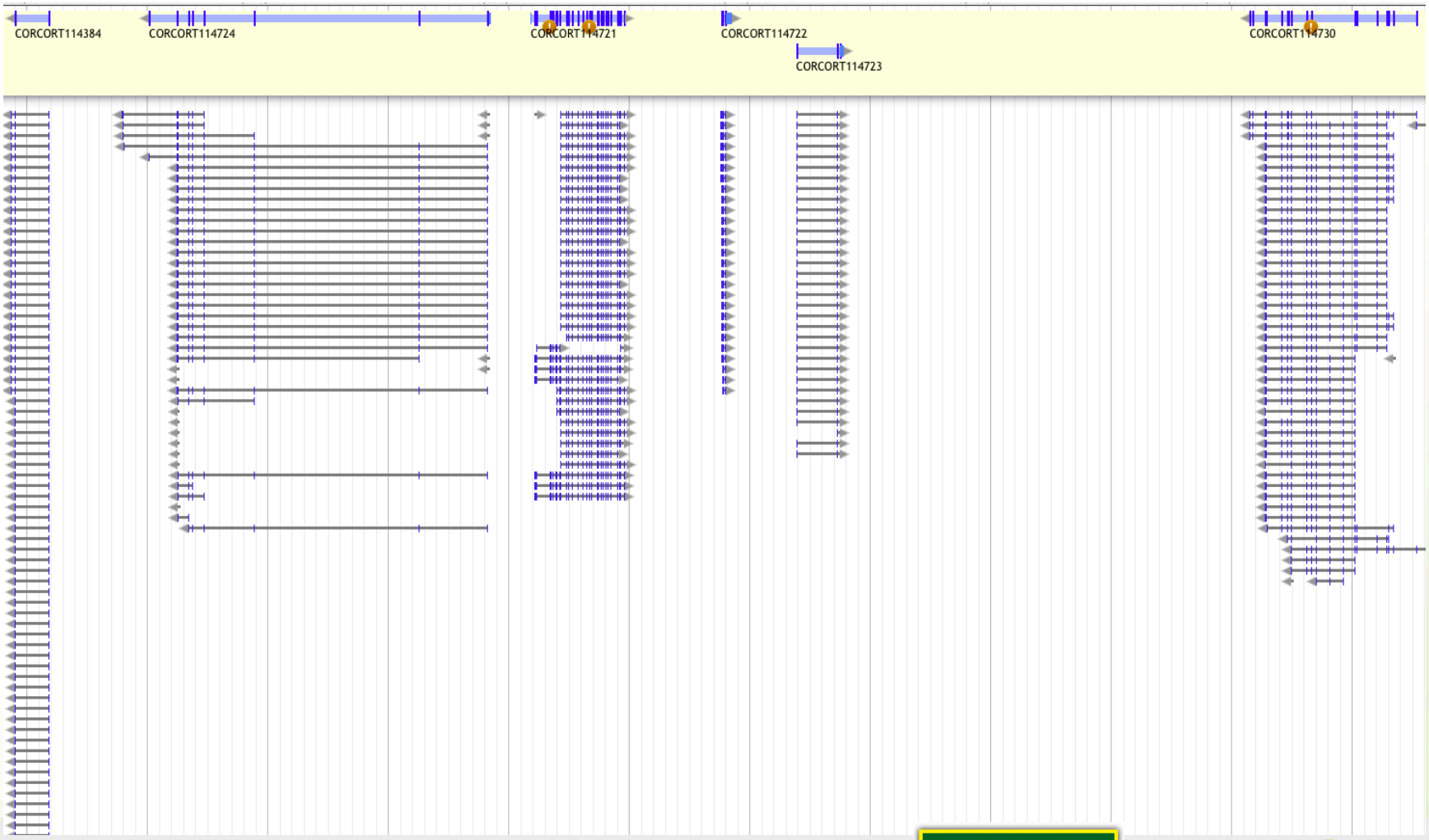
Combined annotation

Transcriptomes are different but have their own challenges

- No introns, but where are the start and stop codons?
- Still needs functional annotation



Data used - Proteins



Data used - Proteins

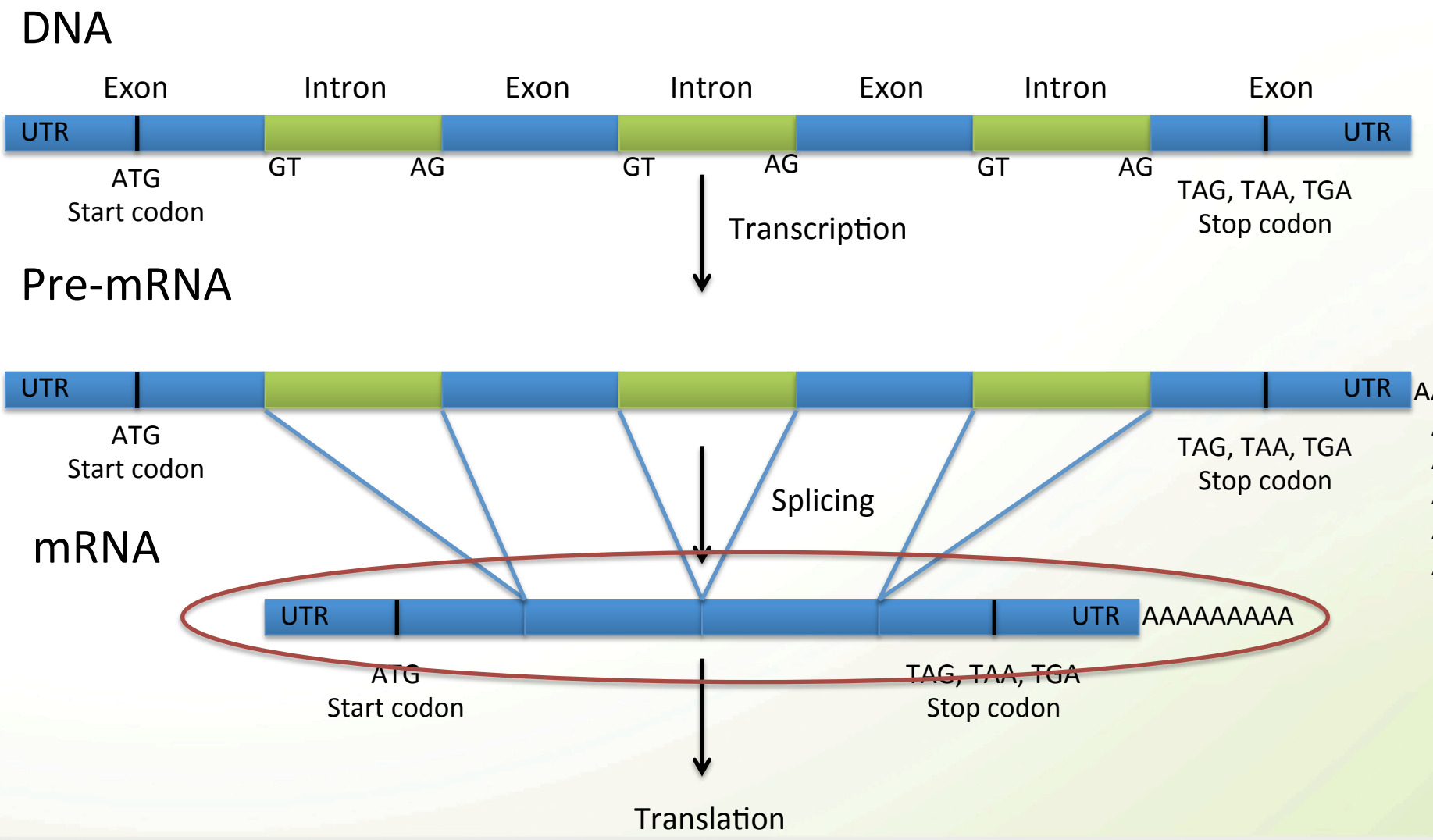
- Conserved in sequence => conserved annotation with little noise
- Proteins from model organisms often used => bias?
- Proteins can be incomplete => problems as many annotation procedures are heavily dependent on protein alignments

>ENSTGUP00000017616 pep:novel chromosome:taeGut3.2.4:8_random:2849599:2959678:-1 gene:ENSTGUG00000017338 transcript:ENSTGUT000000180
RSPNATEYNWHHLRYPKIPERLNPPAAAGPALSTAEGWMLPWGNGQHPLLARAPGKGRER
DGKELIKKPKTFKFTFLKKKKKKKKKTFK
>ENSTGUP00000017615 pep:novel chromosome:taeGut3.2.4:23_random:205321:209117:1 gene:ENSTGUG00000017337 transcript:ENSTGUT00000018017
PDLRELVLMFEHLHRVRNGGFRNSEVKKWPDRSPPPYHSFTPAQKSFSLAGCSGESTKMG
IKERMRLSSSQRQGSRGRQQHLGPPLHRSPSPEDVAEATSPTKVQKSWSFNDRTRFRASL
RLKPRIPAEGDCPPEDSGEERSSPCDLTFEDIMPAVKTLIRAVRILKFLVAKRKFKETLR
PYDVKDVIEQYSAGHLDMLGRIKSLQTRVEQIVGRDRALPADKKVREKGEKPALEAELVD
ELSMMGRVVKVERQVQSIEHKLDLLLGLYSRCLRKGSANSLVLAAVRVPPGEPDVTSDYQ
SPVEHEDISTSAQSLSISRLASTNMD

Data used - Proteins

- Maker will align proteins for you: Blast -> Exonerate
- Blast is not structure aware, Exonerate is (splice sites, start/stop codons)
- Preferred file-format: fasta

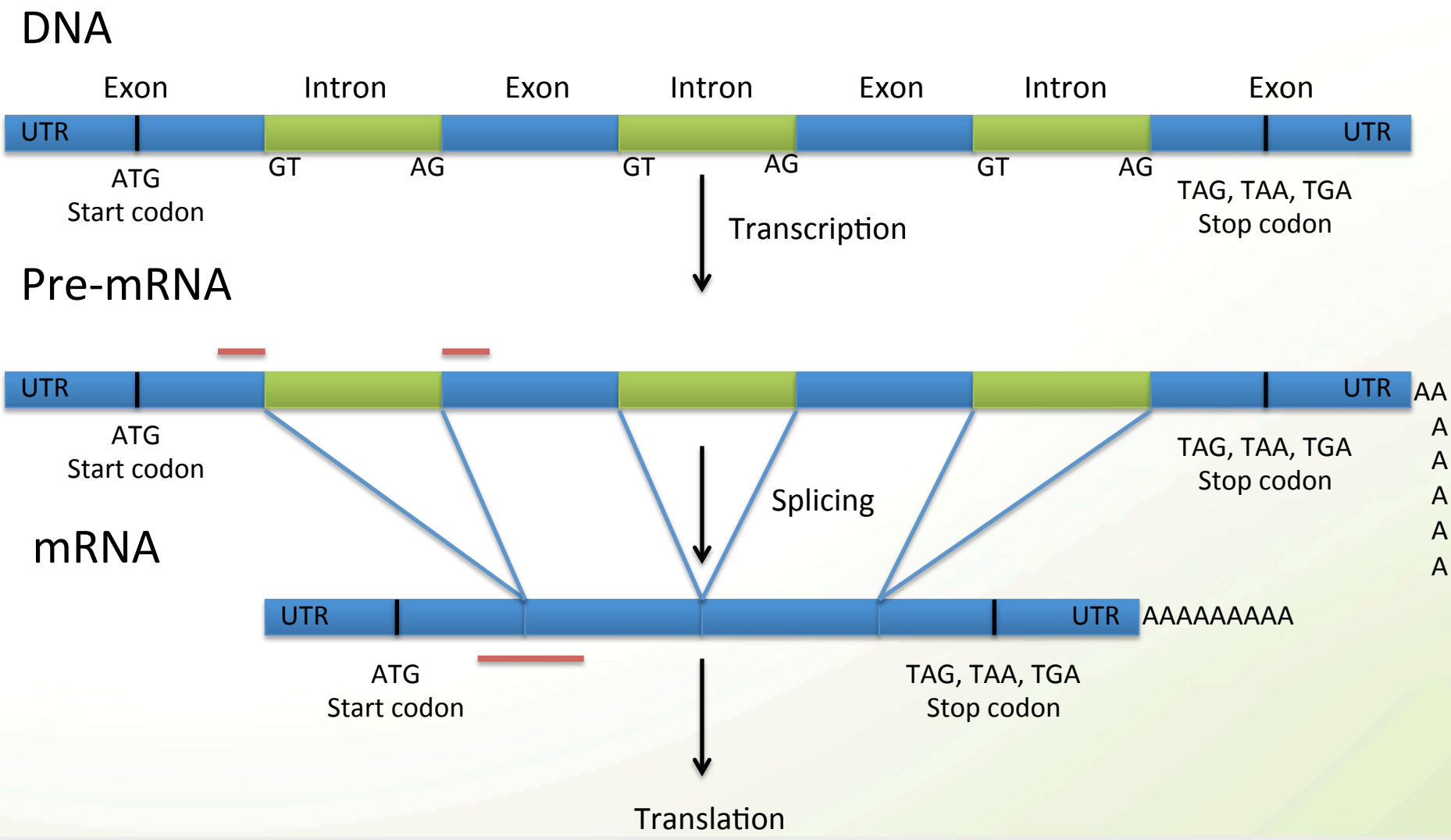
RNA-seq


DNA
Exon
Intron
Exon
Intron
Exon
Intron
Exon
UTR
UTR
GT
AG
GT
AG
GT
AG
ATG
Start codon
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

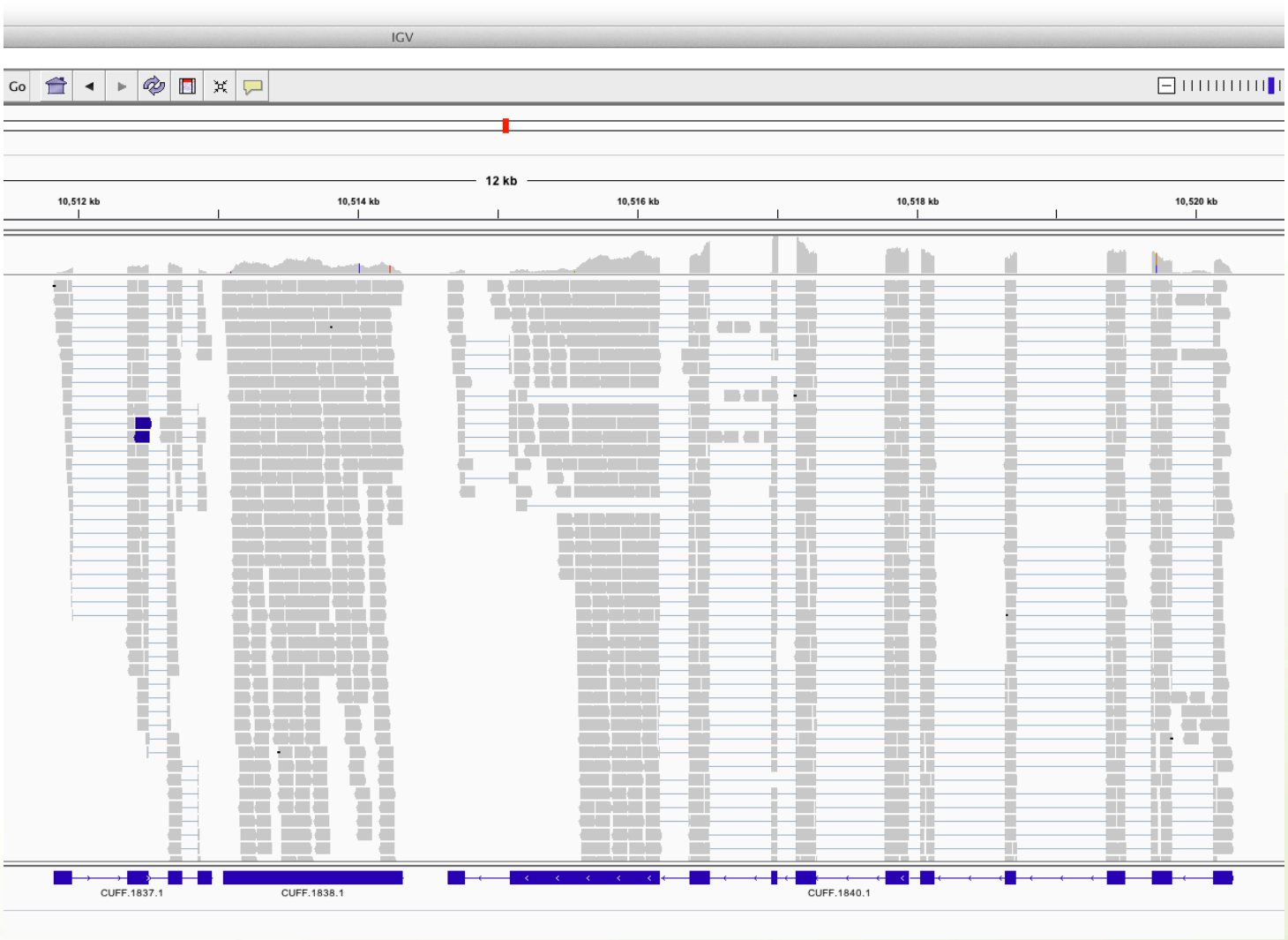
Data used - RNA-seq

- Should always be included in an annotation project
- From the same organism as the genomic data => unbiased
- Can be very noisy (tissue/species dependent), can include pre-mRNA
- PASA, or some other filtering method, often needed

Spliced reads

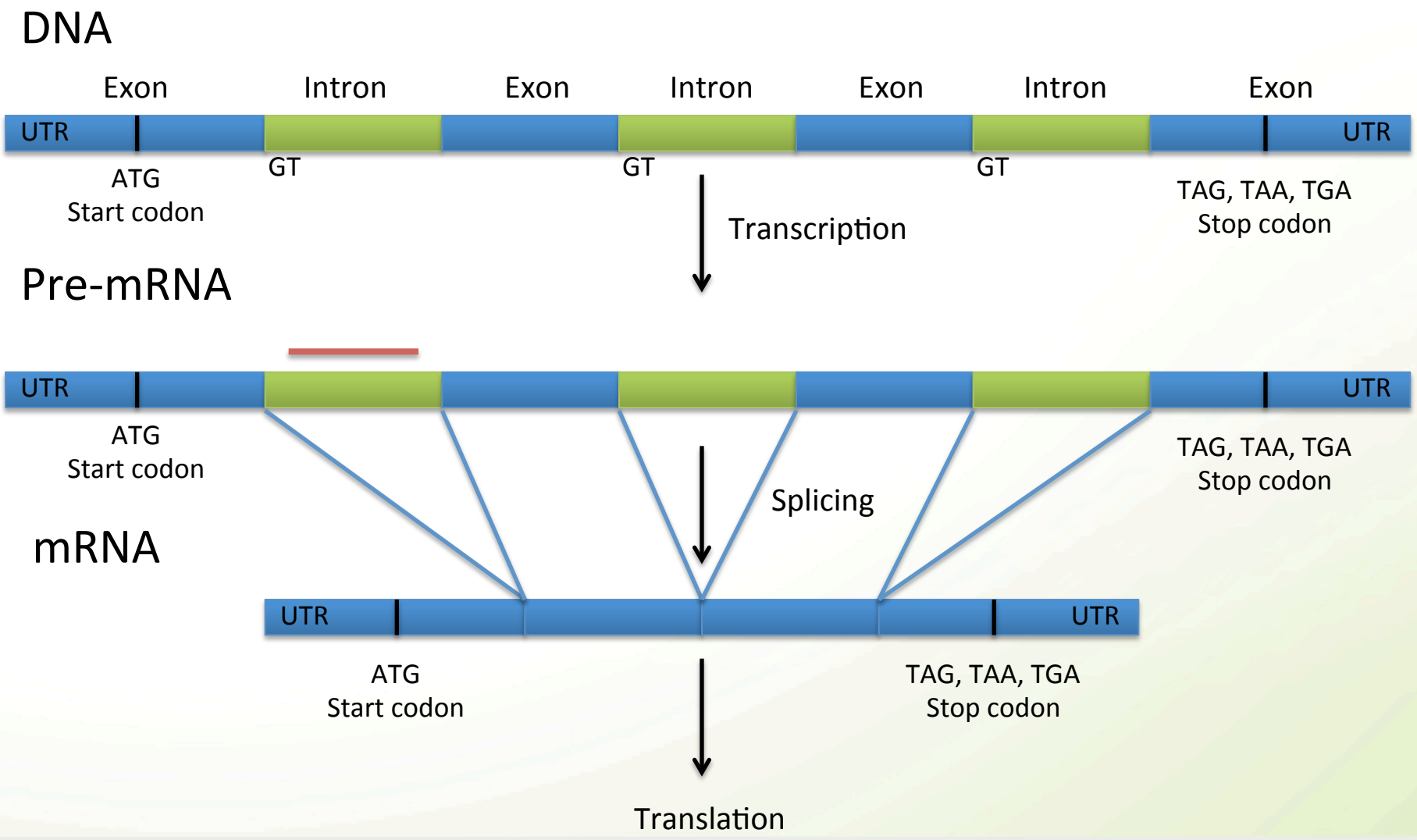


RNA-seq - Spliced reads

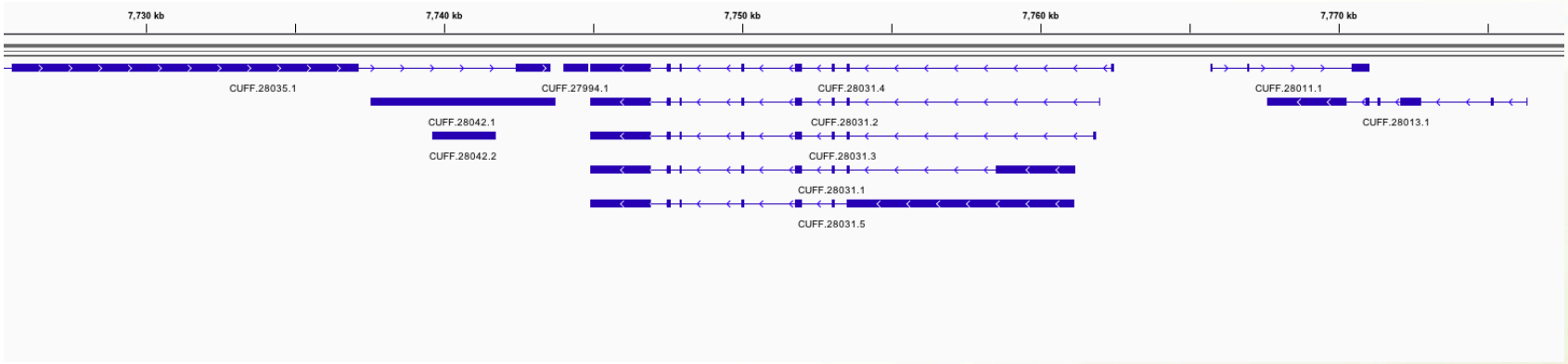


Pre-mRNA

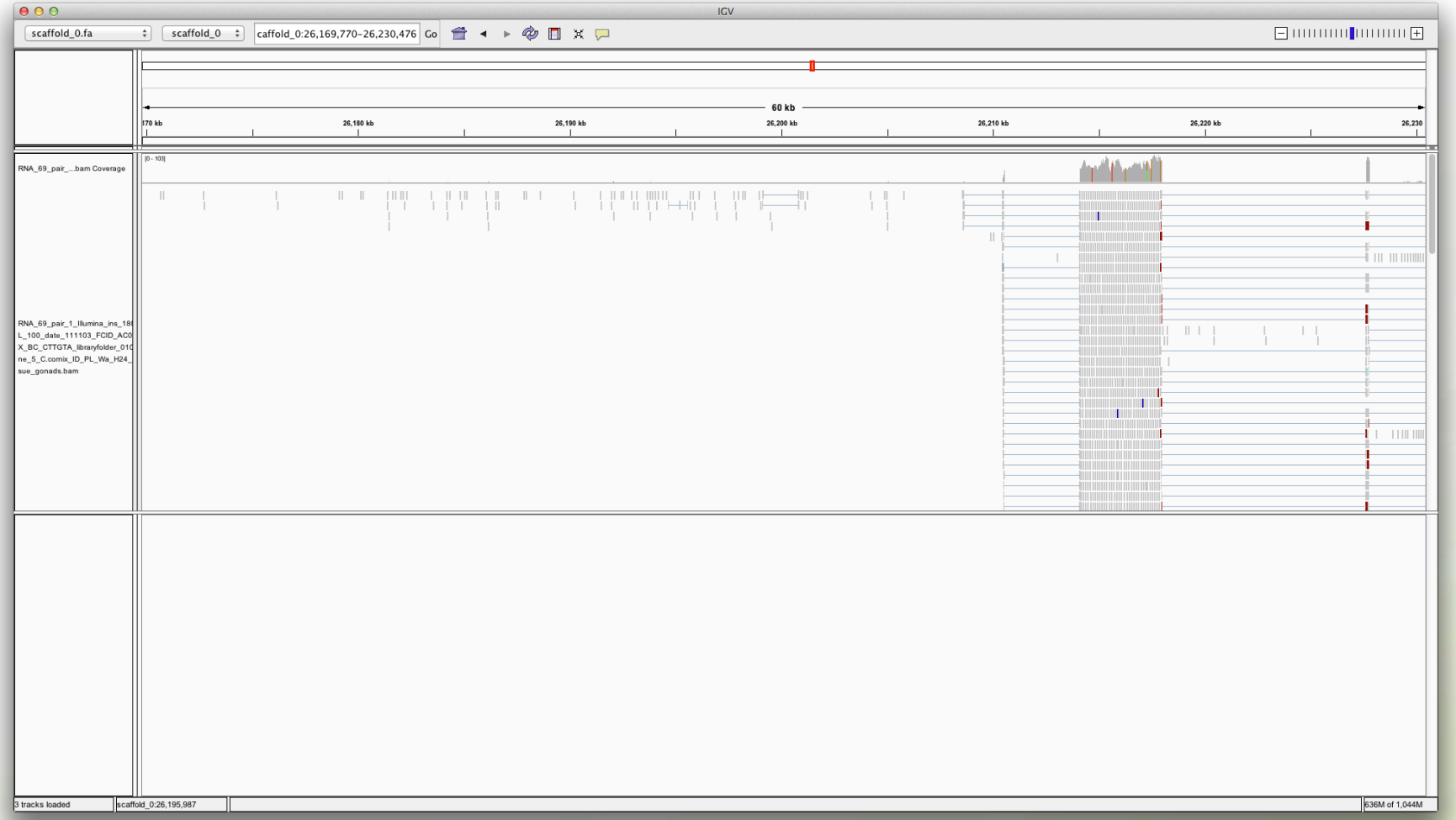
Pre-mRNA



Pre-mRNA

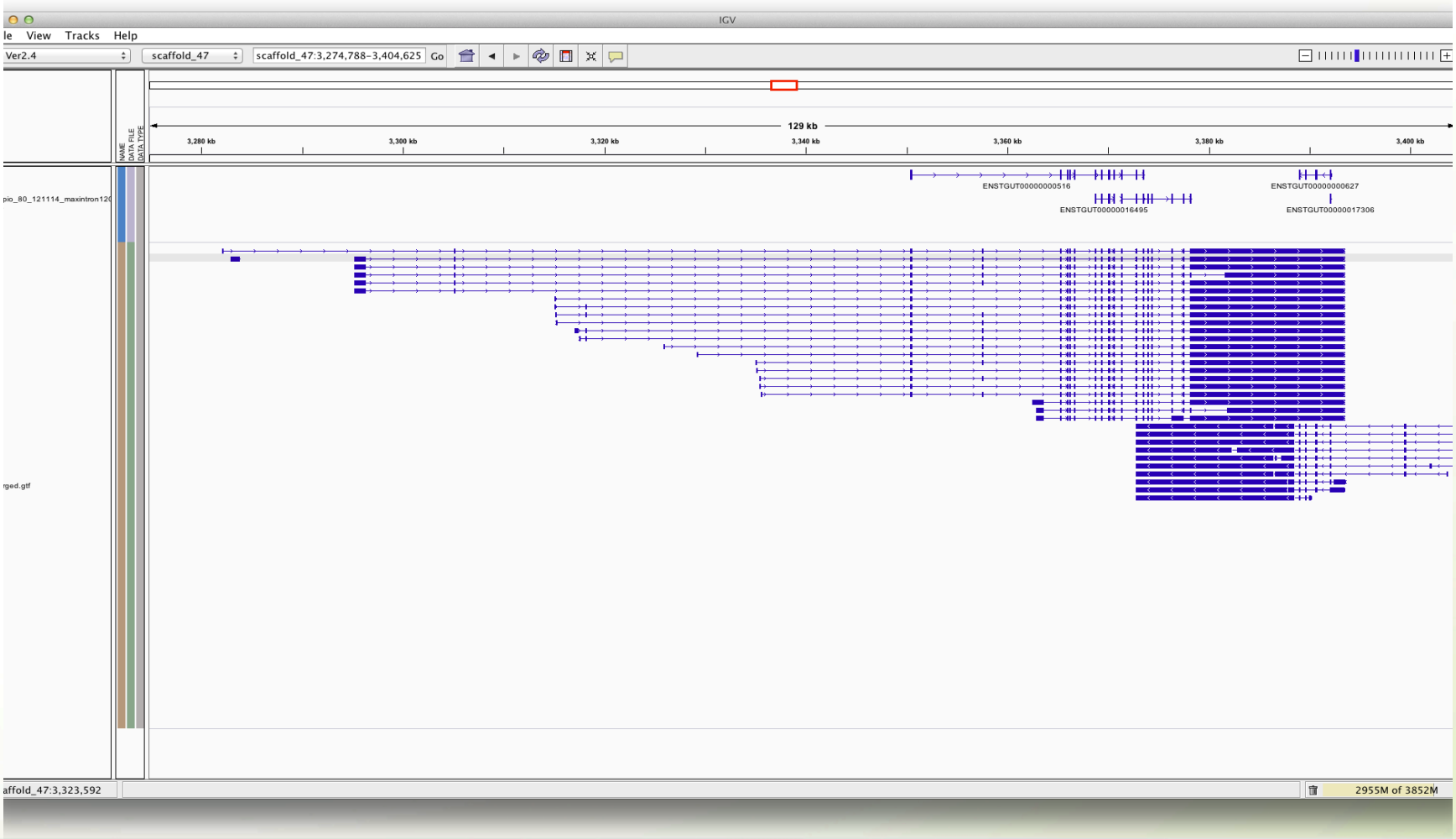

7,730 kb
7,740 kb
7,750 kb
7,760 kb
7,770 kb
CUFF.28035.1
CUFF.27994.1
CUFF.28031.4
CUFF.28011.1
CUFF.28042.1
CUFF.28031.2
CUFF.28013.1
CUFF.28042.2
CUFF.28031.3
CUFF.28031.1
CUFF.28031.5

Includes everything that is transcribed



Stranded rna-seq

Three-prime bias in polyA-selected rna-seq



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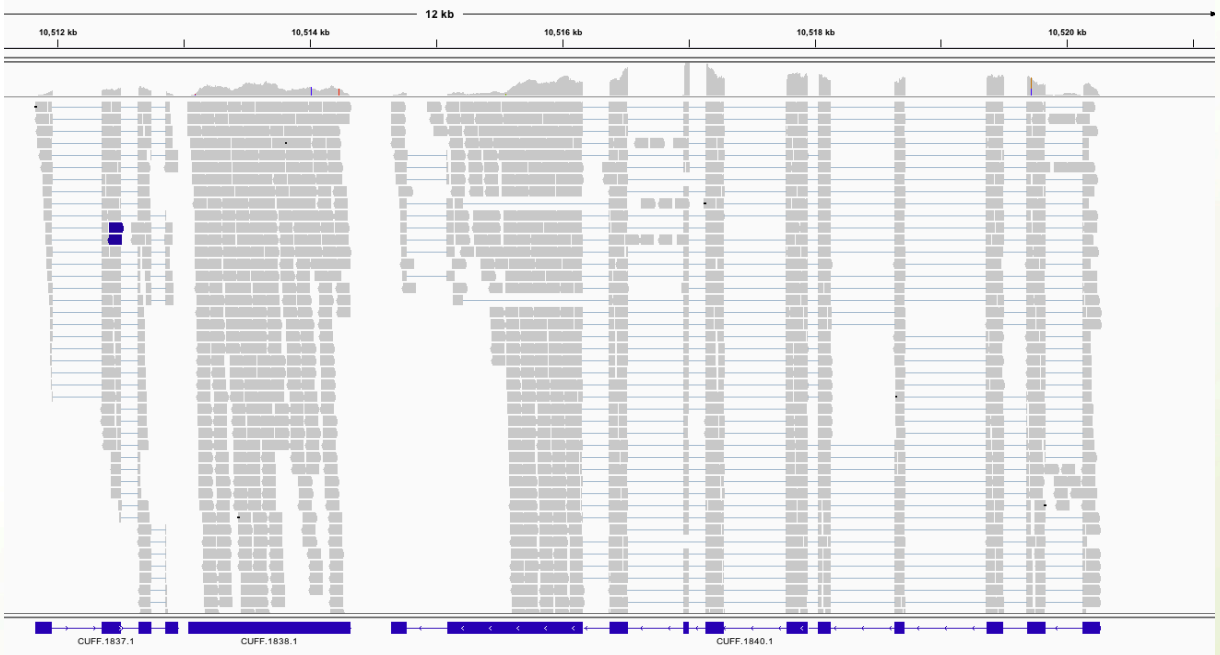
How to use RNA-seq

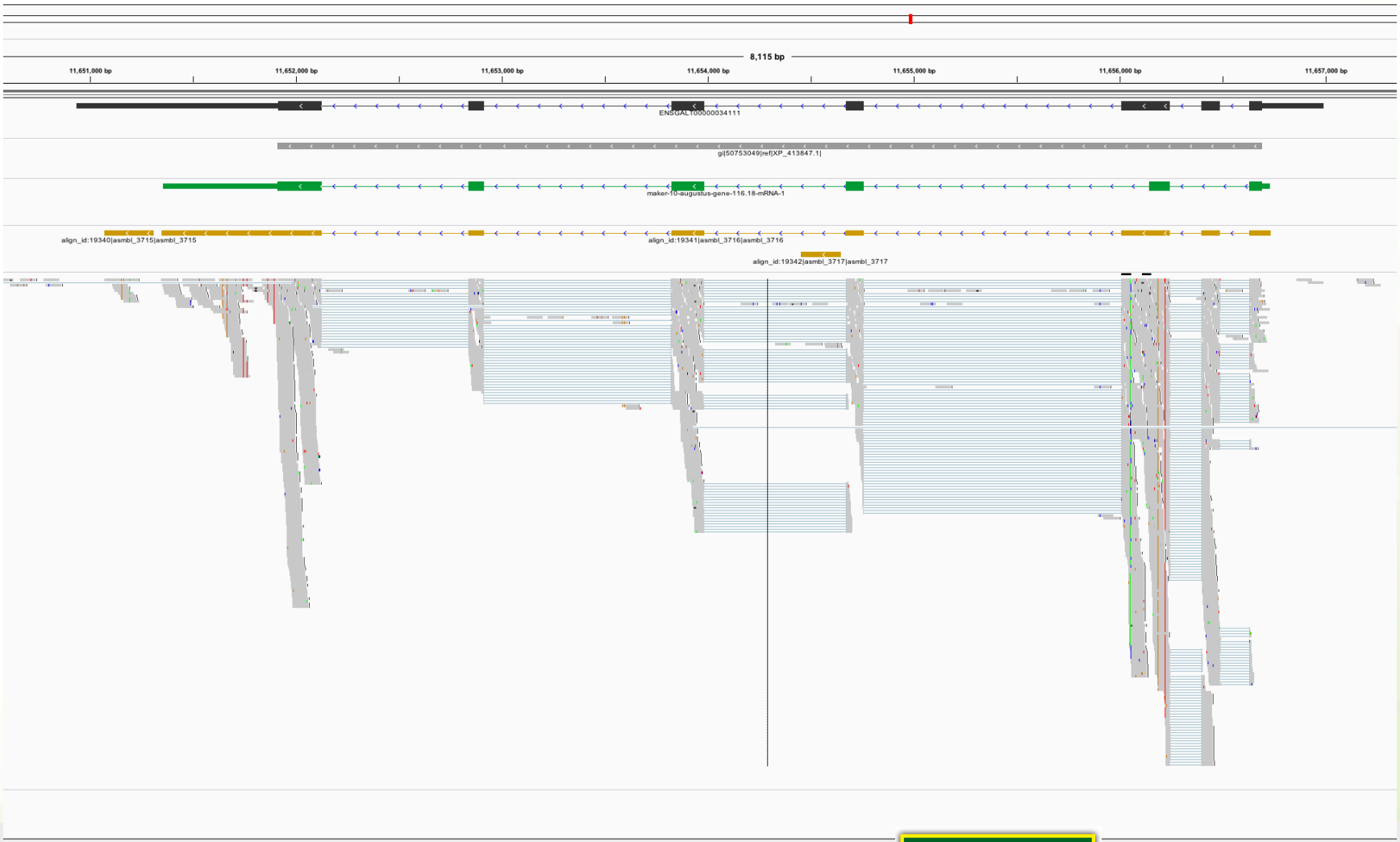
- Maker will align transcripts (ESTs), but these need to be assembled first.
- Cufflinks: mapped reads -> transcripts



How to use RNA-seq

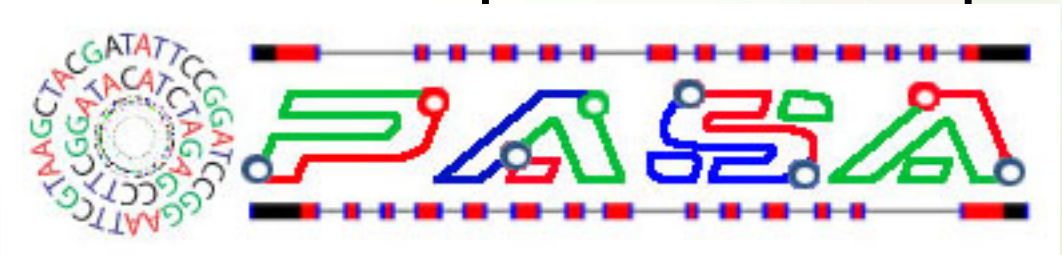
- Maker will align transcripts (ESTs), but these need to be assembled first.
- Cufflinks: mapped reads -> transcripts
- Trinity: assembles transcripts without a genome

Mapped Trinity-assembled transcripts



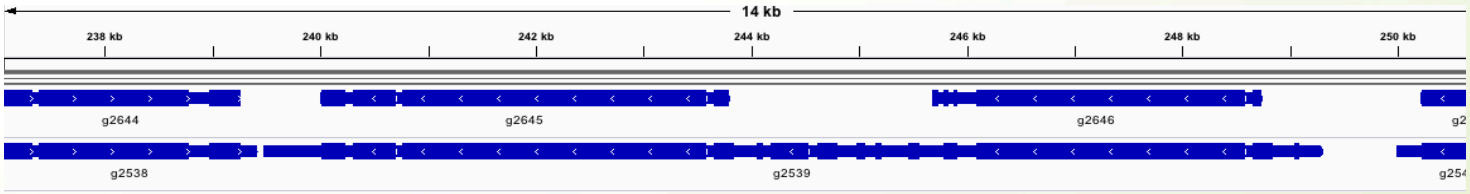
How to use RNA-seq

- Maker will align transcripts (ESTs), but these need to be assembled first.
- Cufflinks: mapped reads -> transcripts
- Trinity: assembles transcripts without a genome
- PASA can be used to improve transcript quality



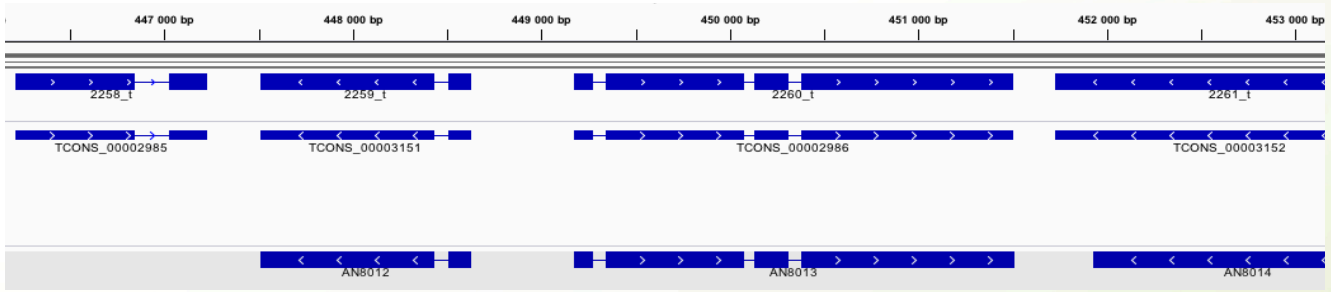
Ab initio gene finders are used in Maker

- Commonly used programs: Augustus, Snap, Genemark-ES, FGENESH, Genscan, Glimmer-HMM,…
- Uses HMM-models to figure out how introns, exons, UTRs etc. are structured
- These HMM-models need to be trained!



Liftovers are very useful for orthology determination

- Kraken
- Align the two genomes (Satsuma) and then transfer annotations between aligned regions



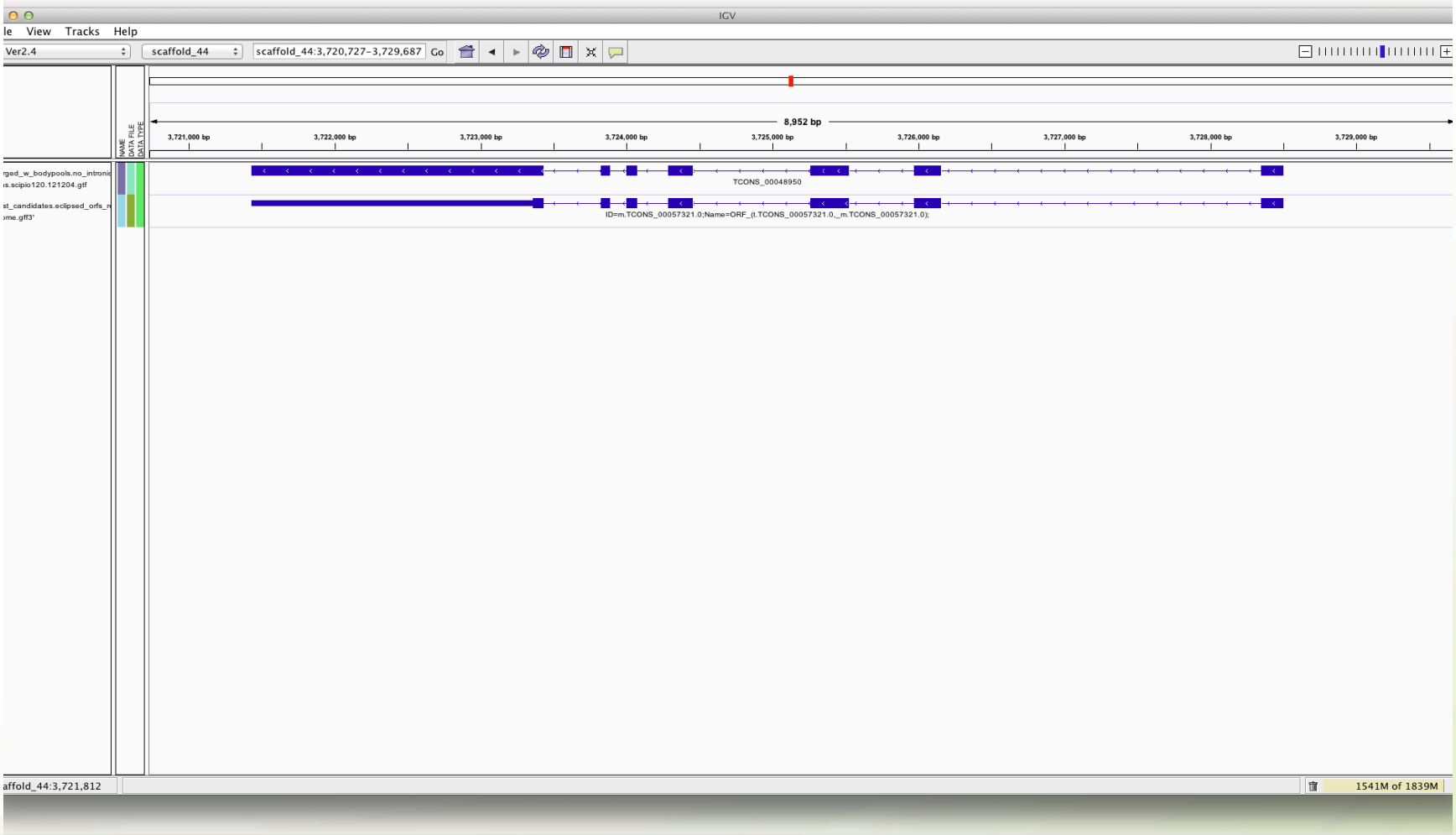
General recommendations

- Always combine different types of evidence!
- One single method is not enough!
- Use Maker!

Transcript annotation

- Here the transcript is already defined. The challenge is to find where the coding regions starts and stops
- Transdecoder

Transdecoder



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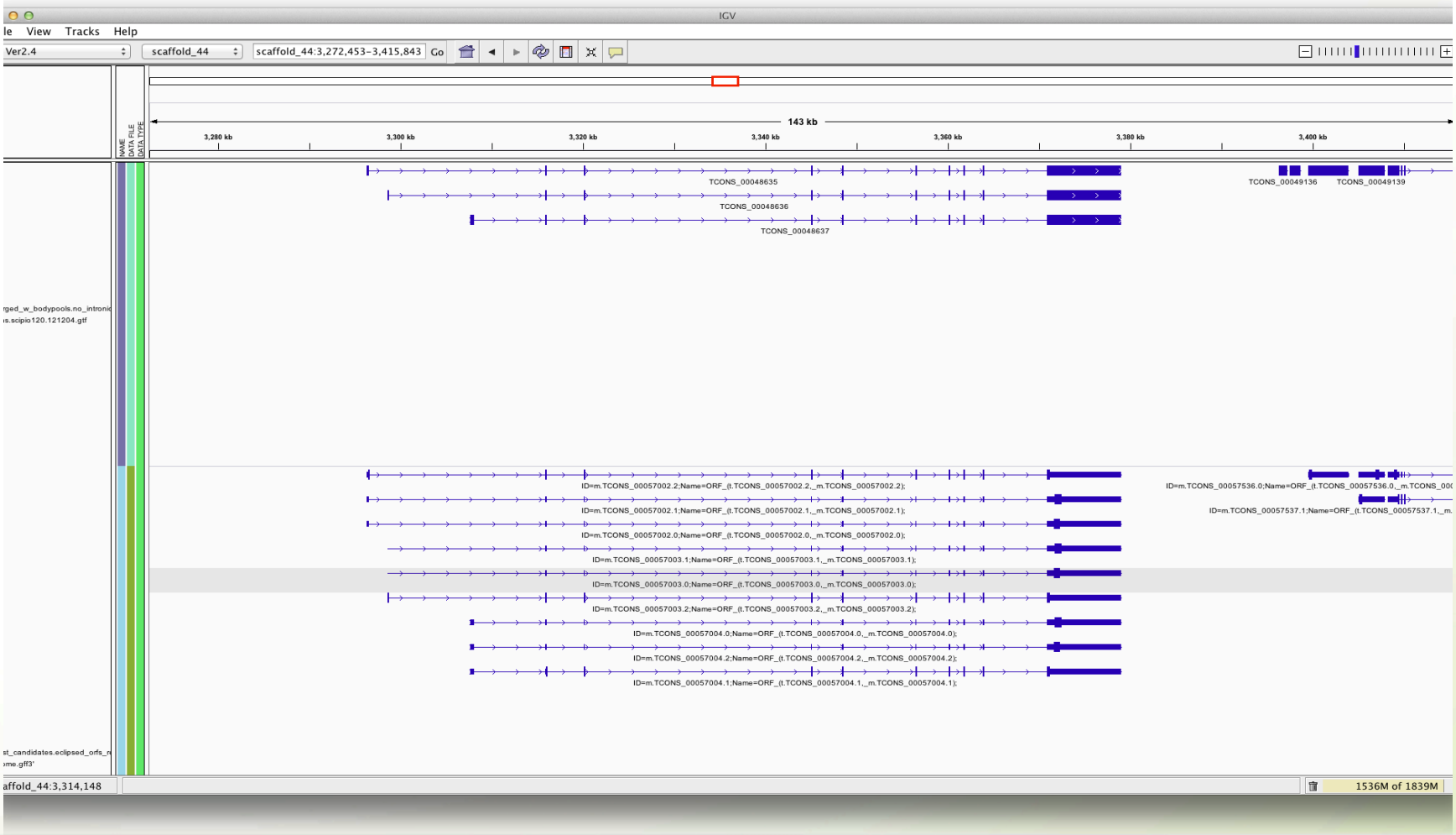
Transdecoder



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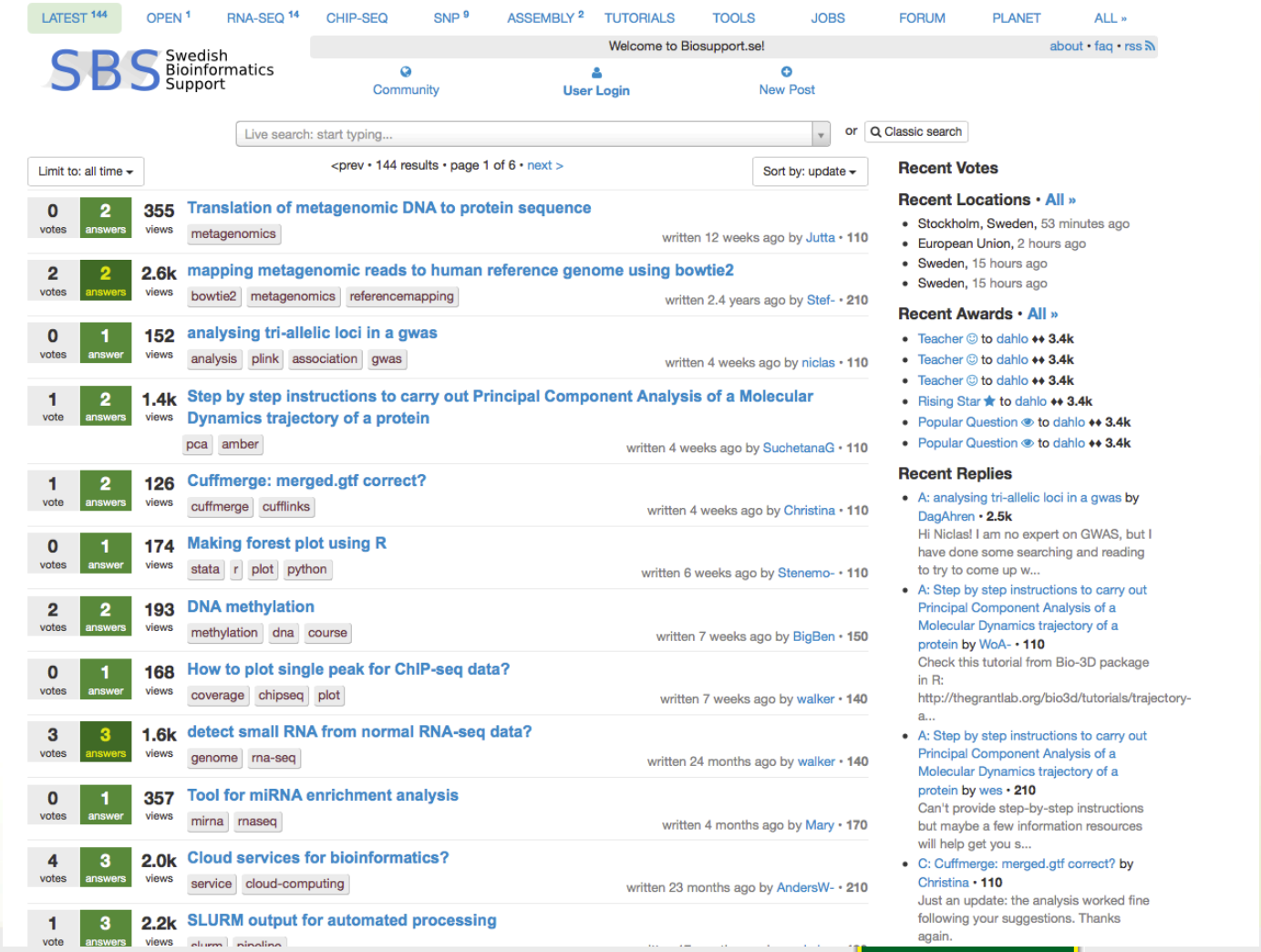
Or get help - BILS assembly and annotation team

- Five people working with assembly and annotation
- Deliver high quality annotations
- Enable visualization and manual curation through a web interface
- Also available for consultation
- support@bils.se

Biosupport.se



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