Genome Literacy Workshop

Elisabeth Busch-Nentwich
& Pavol Kramár

Learning Outcomes

- Understand Ensembl as a database
  - basics of default data
  - investigating homology
- Find and switch on optional features
  - find your gene and its associated data
- Download gene and genome data
  - key tools to use
- Upload and display your own data

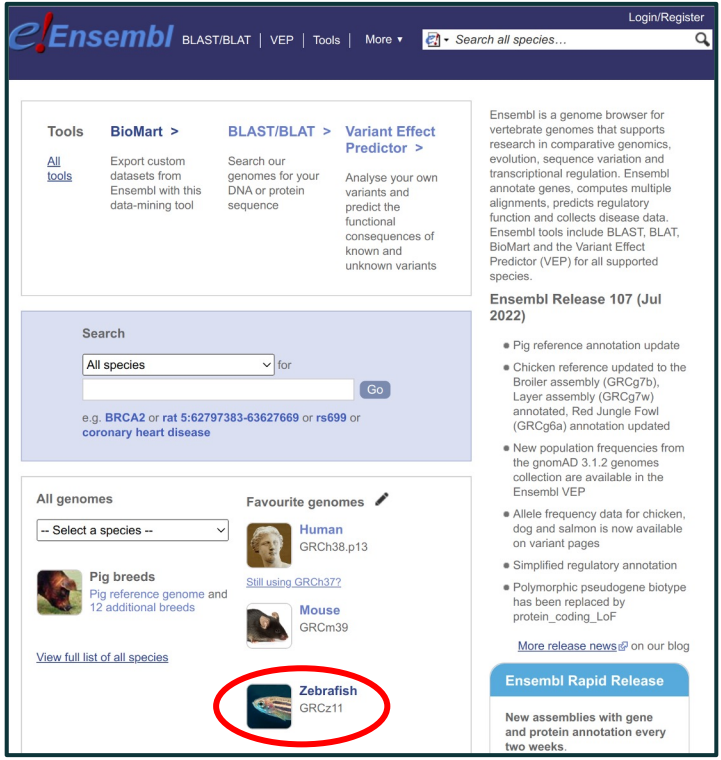
Part 1

- Zebrafish Genome Project
- Ensembl
- Finding your gene
- Gene name and IDs
- Manual and automatic annotation
- Ensembl “Region” view

Ensembl

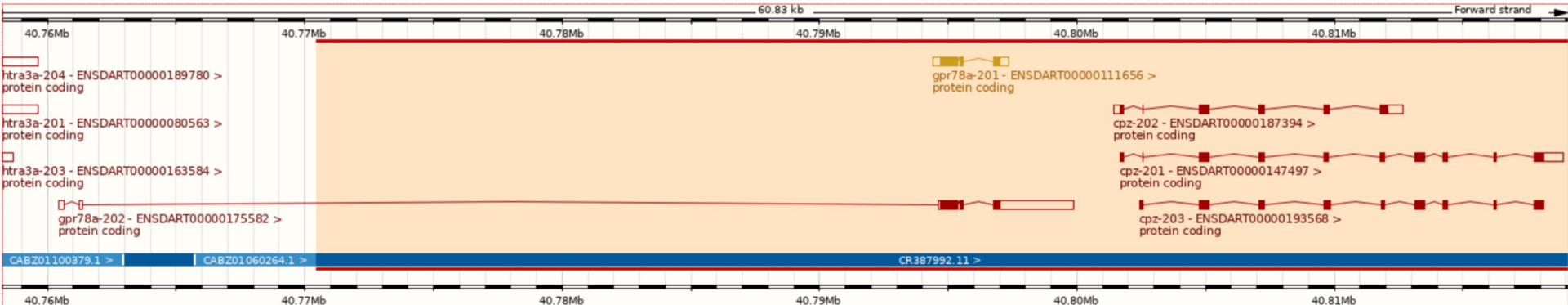
- Most examples from **Ensembl** (we are biased!)
- Probably most widely used genome browser amongst zebrafish researchers
- **Primary source of zebrafish annotation** (UCSC imports Ensembl annotation)

- Currently Ensembl version **109** (Feb 8th)
- New releases 3 or 4 times / year
- Zebrafish **annotation largely static** between releases
- But **naming and homology** updated (+ new functionality)



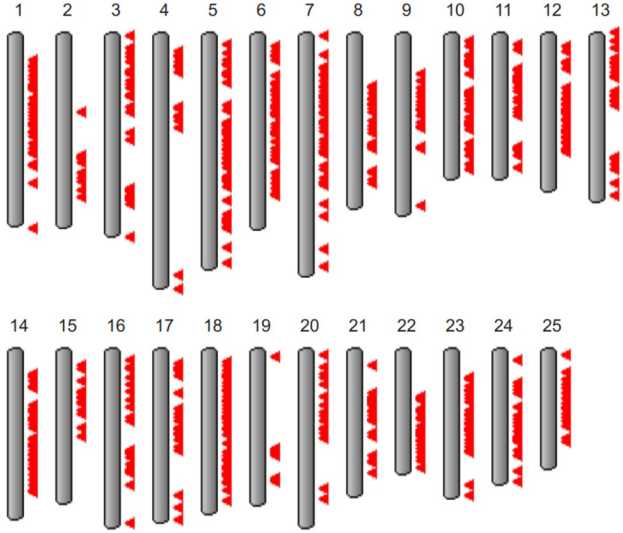
Zebrafish Genome

- **GRCz11** (danRer11) - latest assembly, released in 2017
- Sequencing strategy:
  - 90% clone by clone sequencing
    - **High quality**
  - 10% whole genome shotgun sequencing
    - **Lower quality**
    - Fills gaps between clones
    - Identified by accessions beginning with **CABZ**



Zebrafish Genome History

- Genome project started in **2001** at Sanger Institute
- Initially sequenced pool of **Tübingen** zebrafish
- But zebrafish **very polymorphic** compared to humans
- Too much variation to join clones, so lots of **gaps**
- + same region represented by 2+ clones, leading to **artificial duplication**
- Later used **double haploid** Tübingen fish for some clones and most WGS
- Only **925 gaps** between scaffolds and **N50 > 7 Mbp**
- GRCz11 contains **alternative** scaffolds



From https://www.ncbi.nlm.nih.gov/grc/zebrafish

- When downloading sequence from Ensembl FTP site, "`toplevel`" includes alternative sequence, but "`primary_assembly`" doesn't and is probably what you want

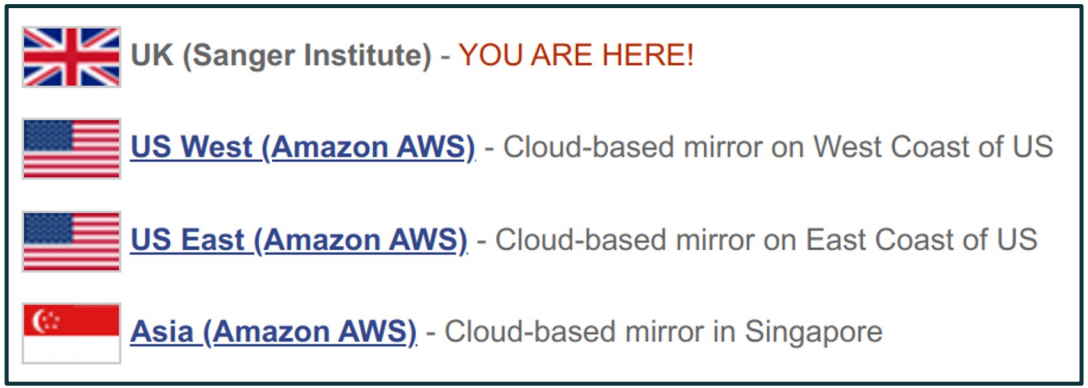
Older Assemblies

- Previous assemblies available in Ensembl **archives**: www.ensembl.org/info/website/archives/assembly.html
  - GRCz10 / danRer10: http://e91.ensembl.org/
  - Zv9 / danRer7: http://e77.ensembl.org/
  - Zv8 / danRer6: http://e54.ensembl.org/

- Even **older** assemblies available in UCSC
- Numbering coordinated when **GRC** (Genome Reference Consortium) took over managing zebrafish assembly from Sanger Institute

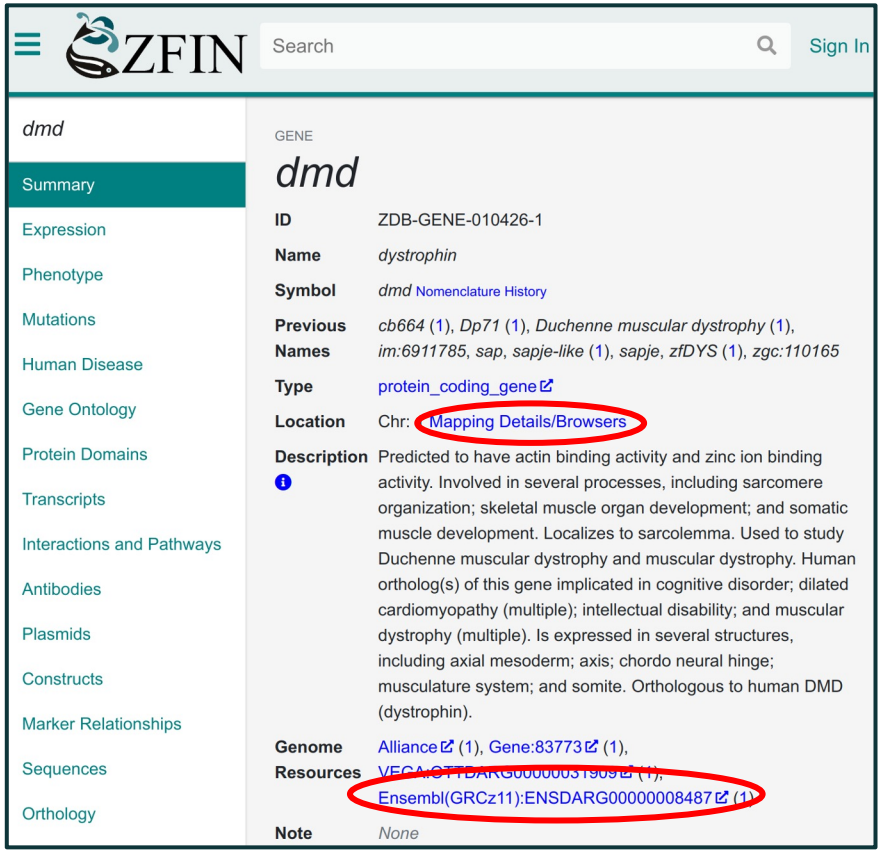
Ensembl Mirrors

- Mirrors: www.ensembl.org/info/about/mirrors.html
- Main site (UK): www.ensembl.org
- US East mirror: useast.ensembl.org
- US West mirror: uswest.ensembl.org
- Most often slow due to chosen tracks though



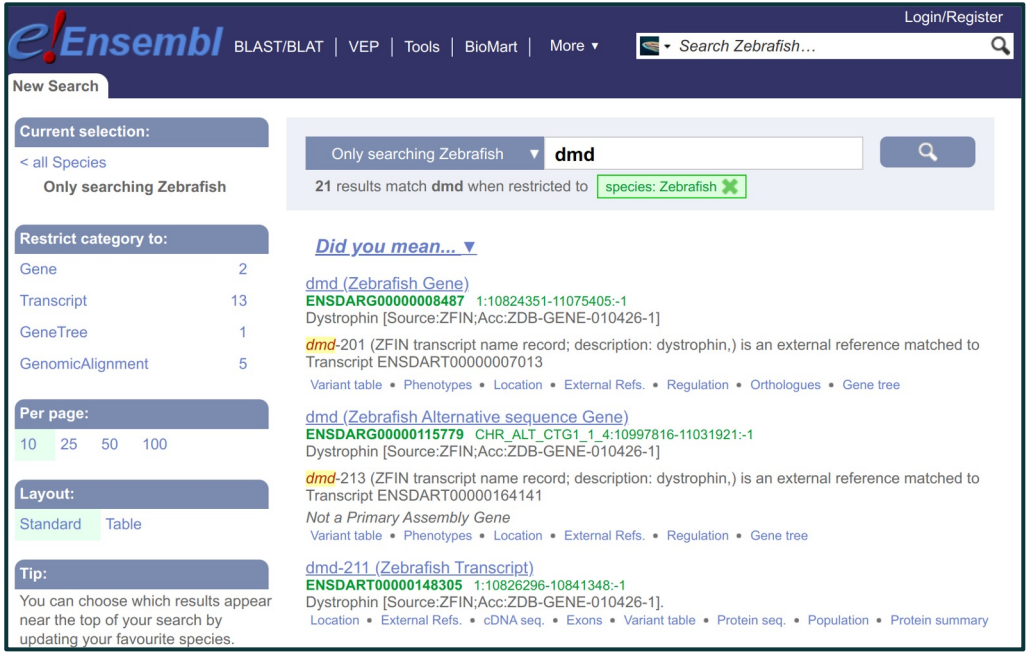
Finding Your Gene

- Follow link from **ZFIN**



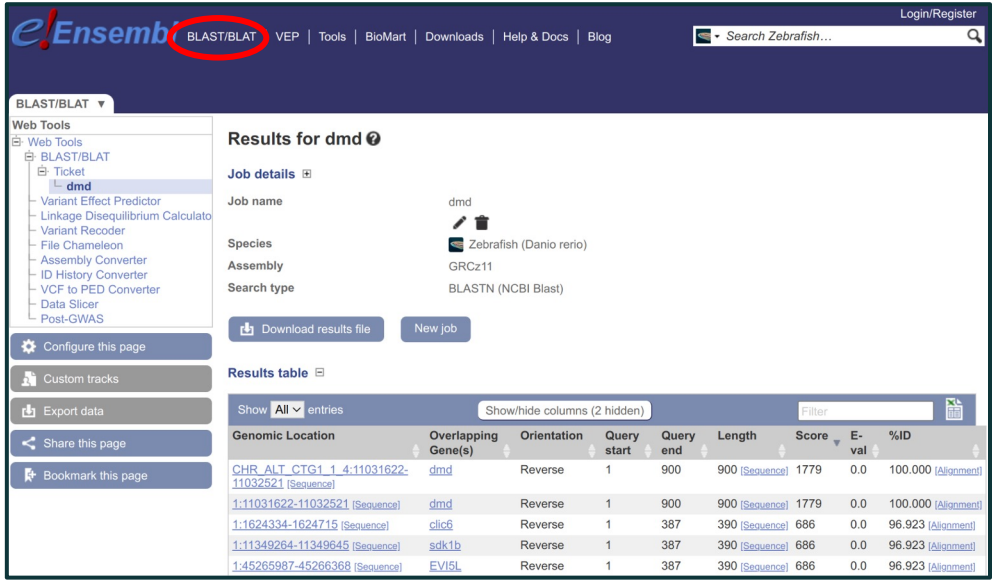
Finding Your Gene

- Follow link from **ZFIN**
- **Search** by gene name on Ensembl (or old name or mutant name)



Finding Your Gene

- Follow link from **ZFIN**
- **Search** by gene name on Ensembl (or old name or mutant name)
- Search using **BLAST** or **BLAT** on Ensembl
  - BLAT is faster
  - BLAST finds more distant alignments + alternative scaffolds
  - No BLAST/BLAT on Ensembl archive sites but can use BLAT on UCSC



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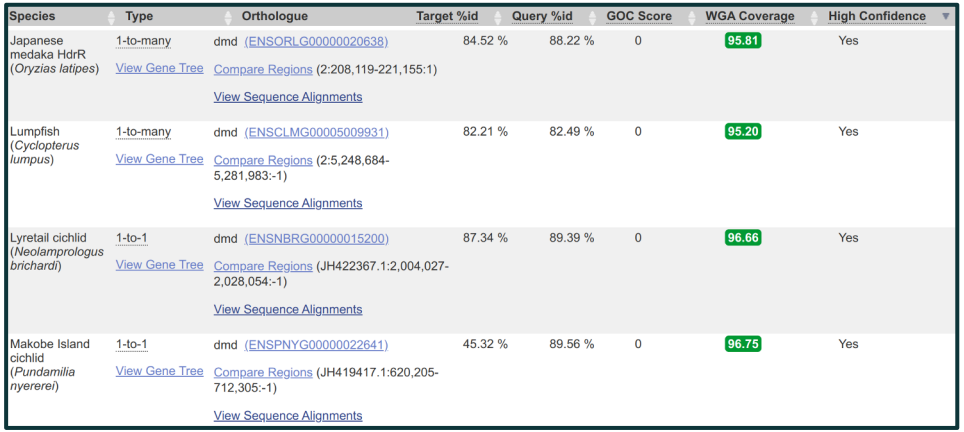
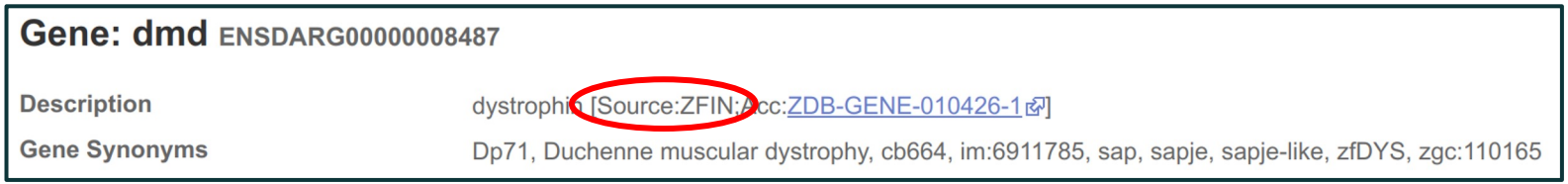
- Check gene correct by checking **orthologues** and/or **synteny**



Species	Type	Orthologue	Target %id	Query %id	GOC Score	WGA Coverage	High Confidence
Japanese medaka HdrR (*Oryzias latipes*)	1-to-many View Gene Tree	dmd (ENSORLG00000020638) Compare Regions (2:208,119-221,155:1) View Sequence Alignments	84.52 %	88.22 %	0	95.81	Yes
Lumpfish (*Cyclopterus lumpus*)	1-to-many View Gene Tree	dmd (ENSCLMG00005009931) Compare Regions (2:5,248,684-5,281,983:-1) View Sequence Alignments	82.21 %	82.49 %	0	95.20	Yes
Lyretail cichlid (*Neolamprologus brichardi*)	1-to-1 View Gene Tree	dmd (ENSNBRG00000015200) Compare Regions (JH422367.1:2,004,027-2,028,054:-1) View Sequence Alignments	87.34 %	89.39 %	0	96.66	Yes
Makobe Island cichlid (*Pundamilia nyererei*)	1-to-1 View Gene Tree	dmd (ENSPNYG00000022641) Compare Regions (JH419417.1:620,205-712,305:-1) View Sequence Alignments	45.32 %	89.56 %	0	96.75	Yes

Gene Names

- Names assigned to Ensembl genes automatically based on **sequence similarity**
  - Mistakes are possible
  - Names can change
- **ZFIN gene symbols** (i.e. the name assigned by ZFIN) are preferred (>23,000 genes), but other databases are also used, e.g. HGNC for ~150 genes, miRBase for ~300 genes
- Description indicates source of name
- Genes without a match are given a name based on the sequence used to identify them, e.g AL645792.1 (clone) or **CABZ**01052570.1 (WGS)



Stable IDs

- Best to use stable IDs
- e.g. **ENS**DARG00000028213 (ttn.2 or ttna)
- **ENS** = Ensembl

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- e.g. ENS**DAR**G00000028213 (ttn.2 or ttna)
- **ENS** = Ensembl
- **DAR** = Danio rerio

Stable IDs

- Best to use stable IDs

- e.g. ENSDAR**G**00000028213 (ttn.2 or ttna)

- **ENS** = Ensembl
- **DAR** = Danio rerio
- **G** = Gene (also T for Transcript, P for Peptide and E for exon)

Stable IDs

- Not completely stable, if annotation or underlying assembly changes
- Stable IDs have **versions**, e.g. ENSDARG00000058767.4
  - Version number of **ENSDARG** increases if transcripts change
  - Version number of **ENSDART** increases if splicing, chromosome or sequence of transcript change
  - Version number of **ENSDARP** increases if peptide's sequence changes
  - Version number of **ENSDARE** increases if exon's sequence changes
- Can also be **removed**, e.g. searching for ENSDARG00000058767

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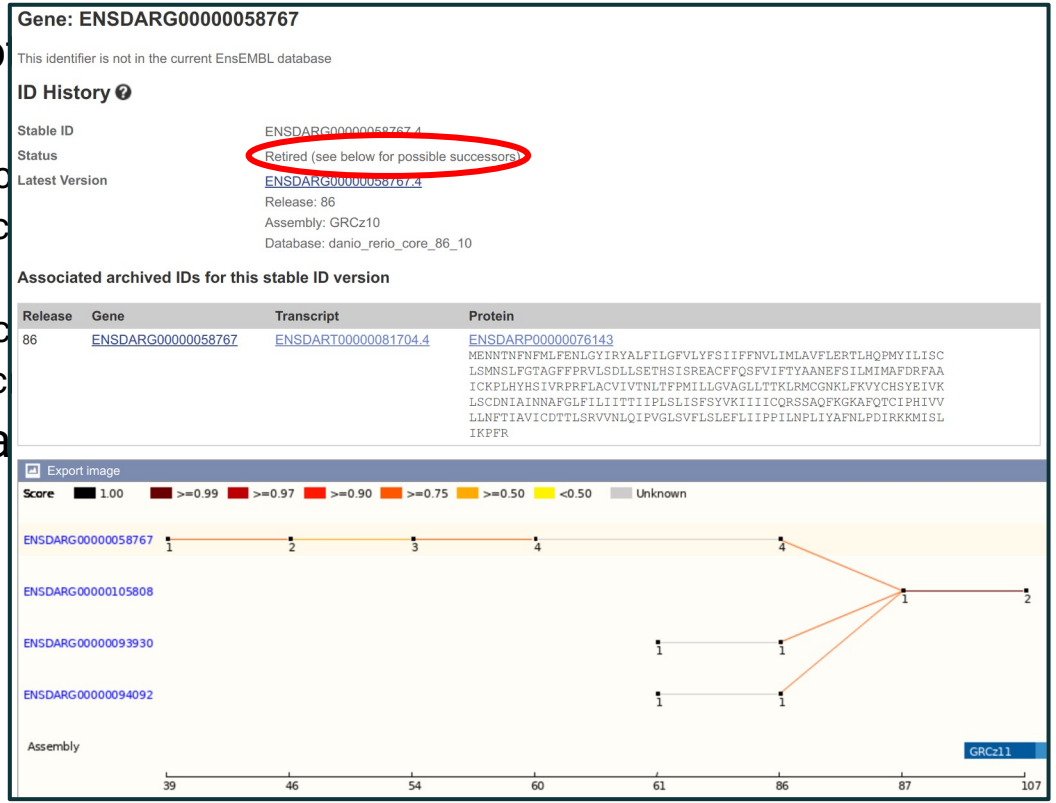
  - Version number of **ENSDART** inc change
  - Version number of **ENSDARP** inc
  - Version number of **ENSDARE** inc
- Can also be **removed**, e.g. sea



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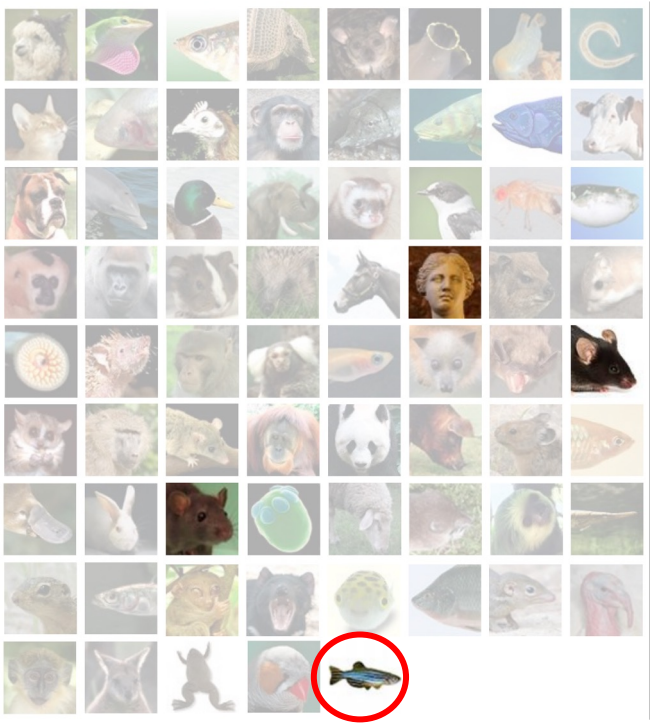
- Can also be **removed**, e.g. searching for ENSDARG00000058767
- Can use www.ensembl.org/Danio_rerio/Tools/IDMapper to convert older IDs to what they **map** to currently in Ensembl

Gene Annotation

- Zebrafish (+ human, mouse, rat) has **manual** and **automatic** gene annotation
- Other **300+** genomes in Ensembl only have automatic annotation
- www.ensembl.org/info/about/species.html



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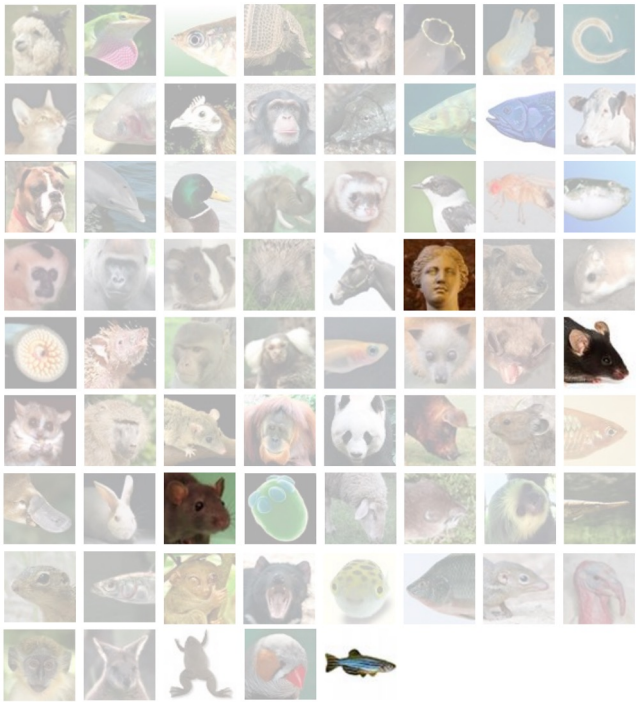
Manual Annotation

- **Gold** standard
- Uses information from databases and publications
- More accurate for tricky areas:
  - e.g. UTRs, splice sites, single exon transcripts
- **Slower** and more expensive
- Thorough, but leads to inclusion of transcripts that may not be representative (e.g. low expression)
- Only clones manually annotated



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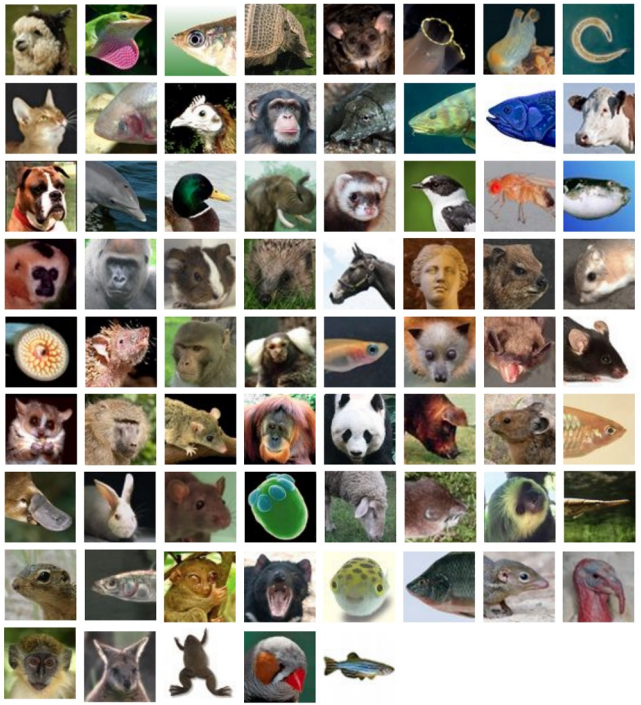
Automatic Annotation

- **Faster**
- Uses evidence from sequences **deposited** in ENA/GenBank/DDBJ and UniProt proteins
- **Overview:**
  - Identify repeats and low complexity sequence with RepeatMasker, Dust and TRF
  - Run GENSCAN to identify *ab initio* gene predictions
  - Align UniProt proteins to GENSCAN predictions, prioritising zebrafish proteins or those from closely related or well annotated species
  - Make gene models using Genewise
  - Align cDNAs, ESTs and RNA-seq to annotate UTRs and make RNA-seq gene models
  - Collapse redundant transcripts and cluster into genes, prioritising manual annotation but including automatic annotation if different splicing
  - Identify pseudogenes by looking for genes with frameshifts / repeats
  - Identify processed pseudogenes by looking for multi-exon equivalent



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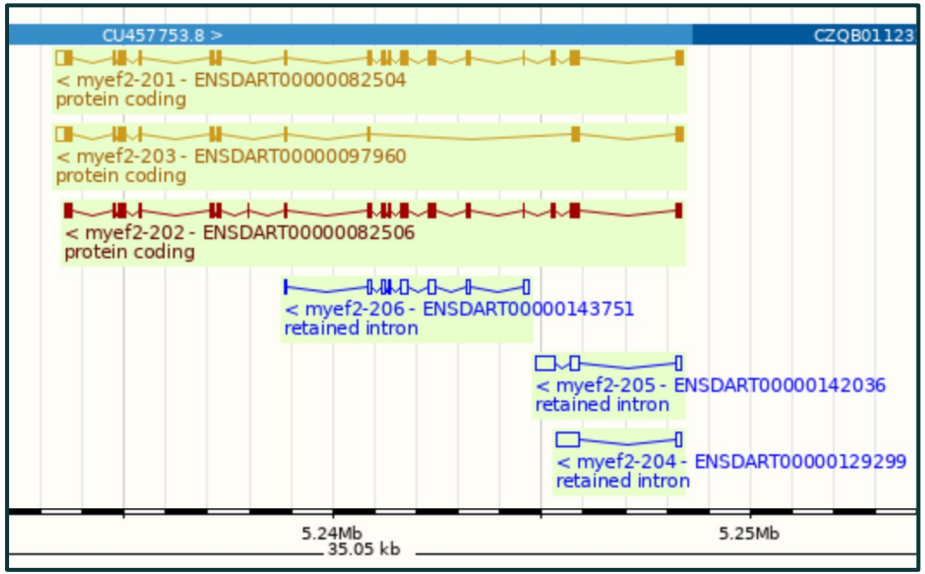
Merged Annotation

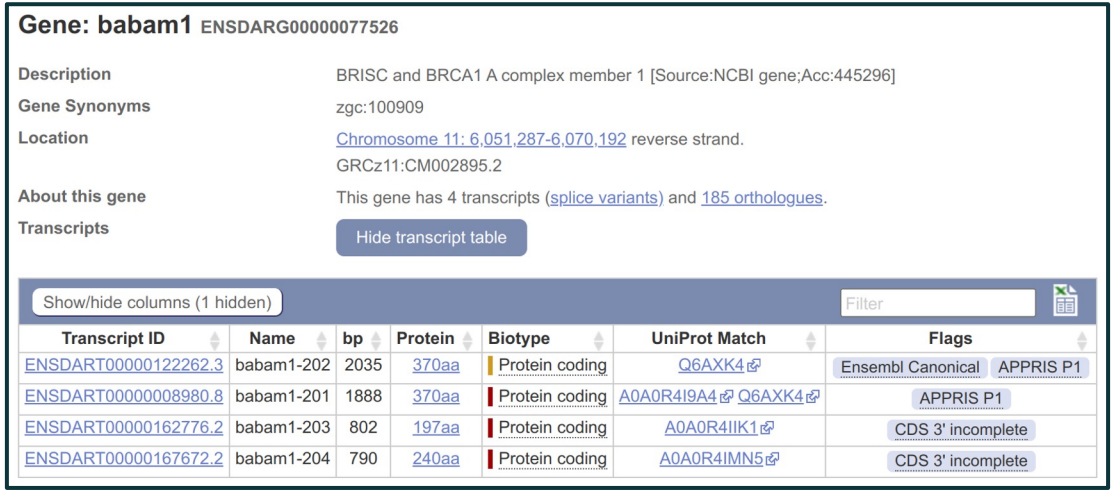
- Golden: **Identical** manual and automatic annotation
- Red: **Protein-coding** transcript from automatic annotation
- Blue: **Non-coding** transcript
- Filled box: **Coding exon**
- Non-filled box: **Non-coding** exon



- In reality, would not trust these retained intron transcripts unless shown to have comparable expression levels

Which Transcript?

- Often **multiple** transcripts
- **Best** transcript for experiments?
- Golden transcript is a good bet
- **Ensembl Canonical** transcript is, on balance, most conserved, most expressed, longest CDS (coding sequence) and in other databases
- APPRIS combines protein structure, important residues and homology to identify a **principal isoform** - APPRIS P1



Gene: babam1 ENSDARG00000077526

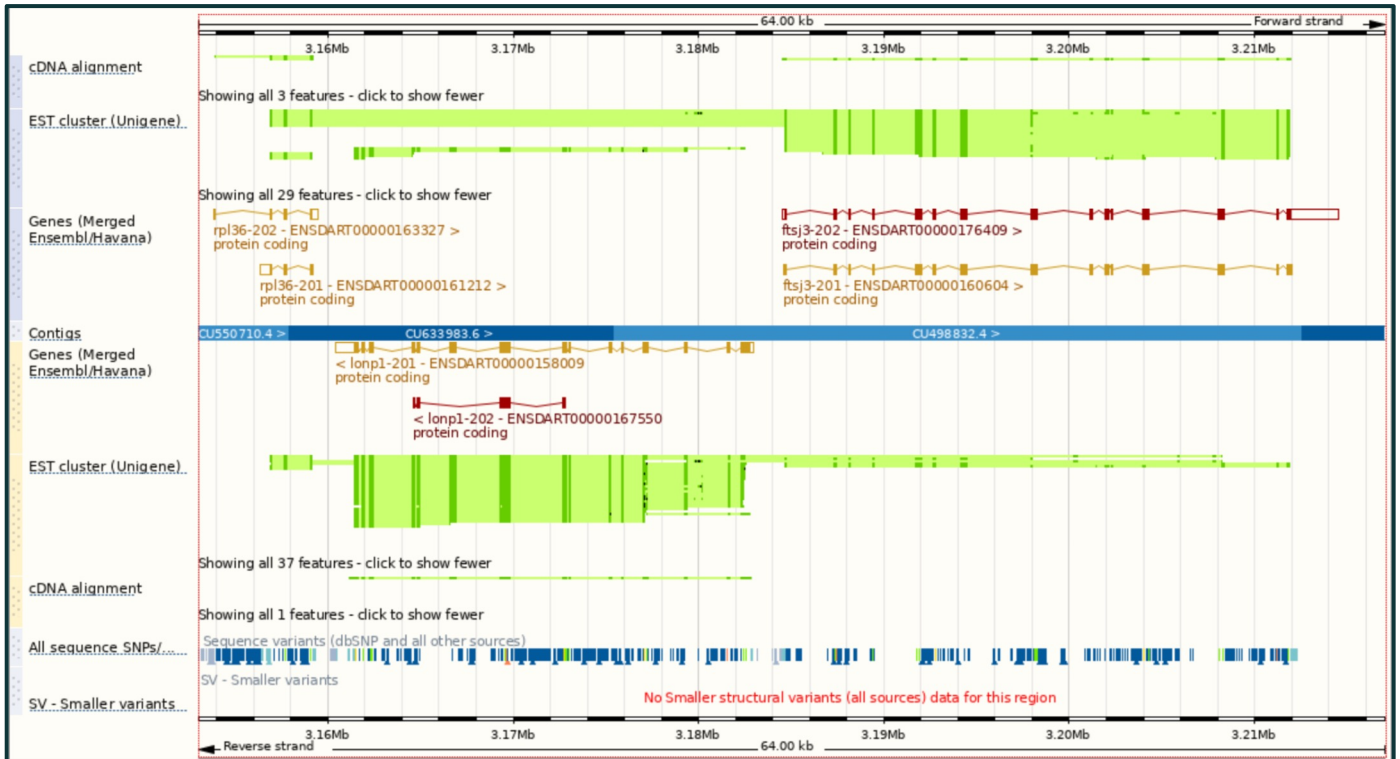
Description	BRISC and BRCA1 A complex member 1 [Source:NCBI gene;Acc:445296]
Gene Synonyms	zgc:100909
Location	Chromosome 11: 6,051,287-6,070,192 reverse strand. GRCz11:CM002895.2
About this gene	This gene has 4 transcripts (splice variants) and 185 orthologues.
Transcripts	Hide transcript table

Show/hide columns (1 hidden) Filter

Transcript ID	Name	bp	Protein	Biotype	UniProt Match	Flags
ENSDART00000122262.3	babam1-202	2035	370aa	Protein coding	Q6AXK4	Ensembl Canonical APPRIS P1
ENSDART00000008980.8	babam1-201	1888	370aa	Protein coding	A0A0R4I9A4 Q6AXK4	APPRIS P1
ENSDART00000162776.2	babam1-203	802	197aa	Protein coding	A0A0R4IIK1	CDS 3' incomplete
ENSDART00000167672.2	babam1-204	790	240aa	Protein coding	A0A0R4IMN5	CDS 3' incomplete

"Region in detail" Demo

- Go to "22:3153000-3217000"



- 4 clones
- 2 genes on +
- 1 gene on -
- Manual + automatic annotation
- cDNA + EST tracks
- Variant tracks

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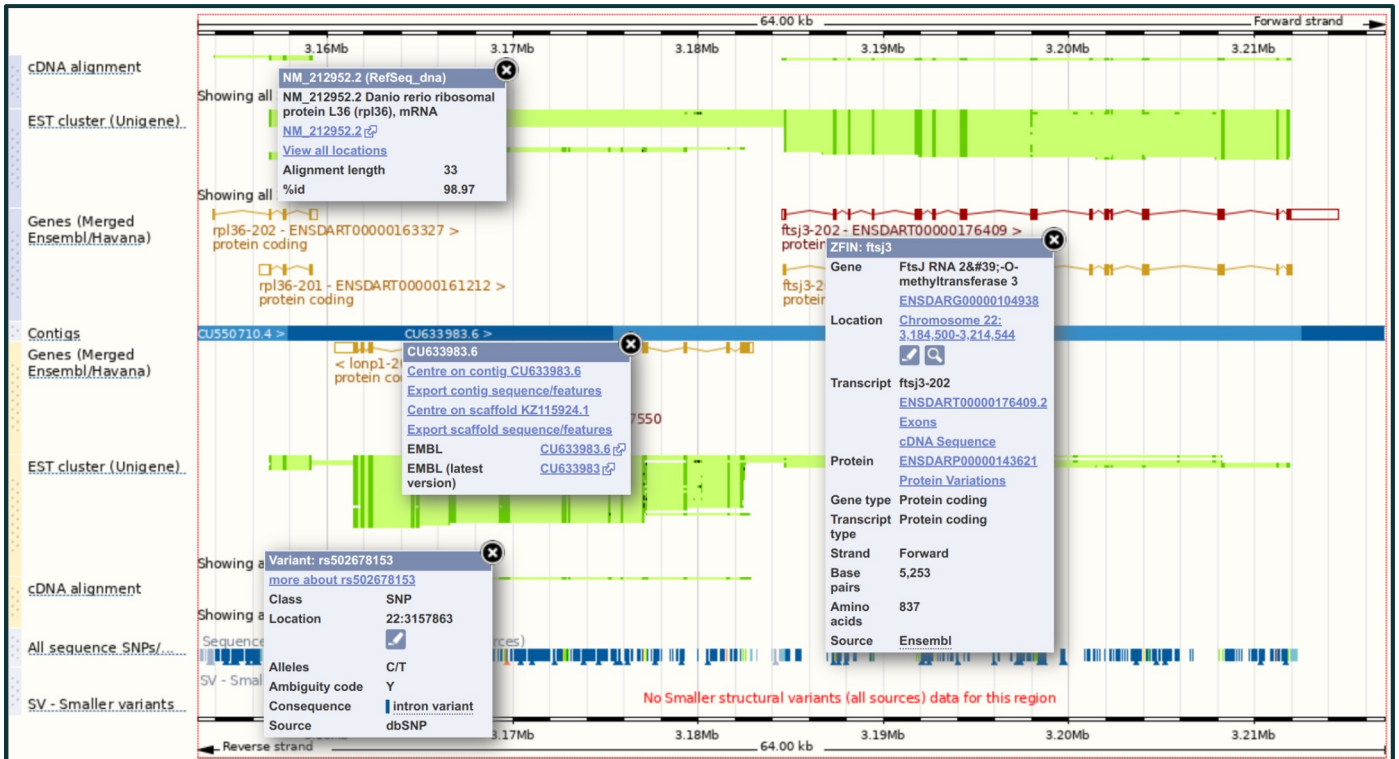

- 4 clones
- 2 genes on +
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Exercise 1

- Do Exercise 1 - “exploring the genome”
- Covers:
  - Region view
  - BLAST/BLAT
  - Archive sites
- Go to mbl2023.buschlab.org

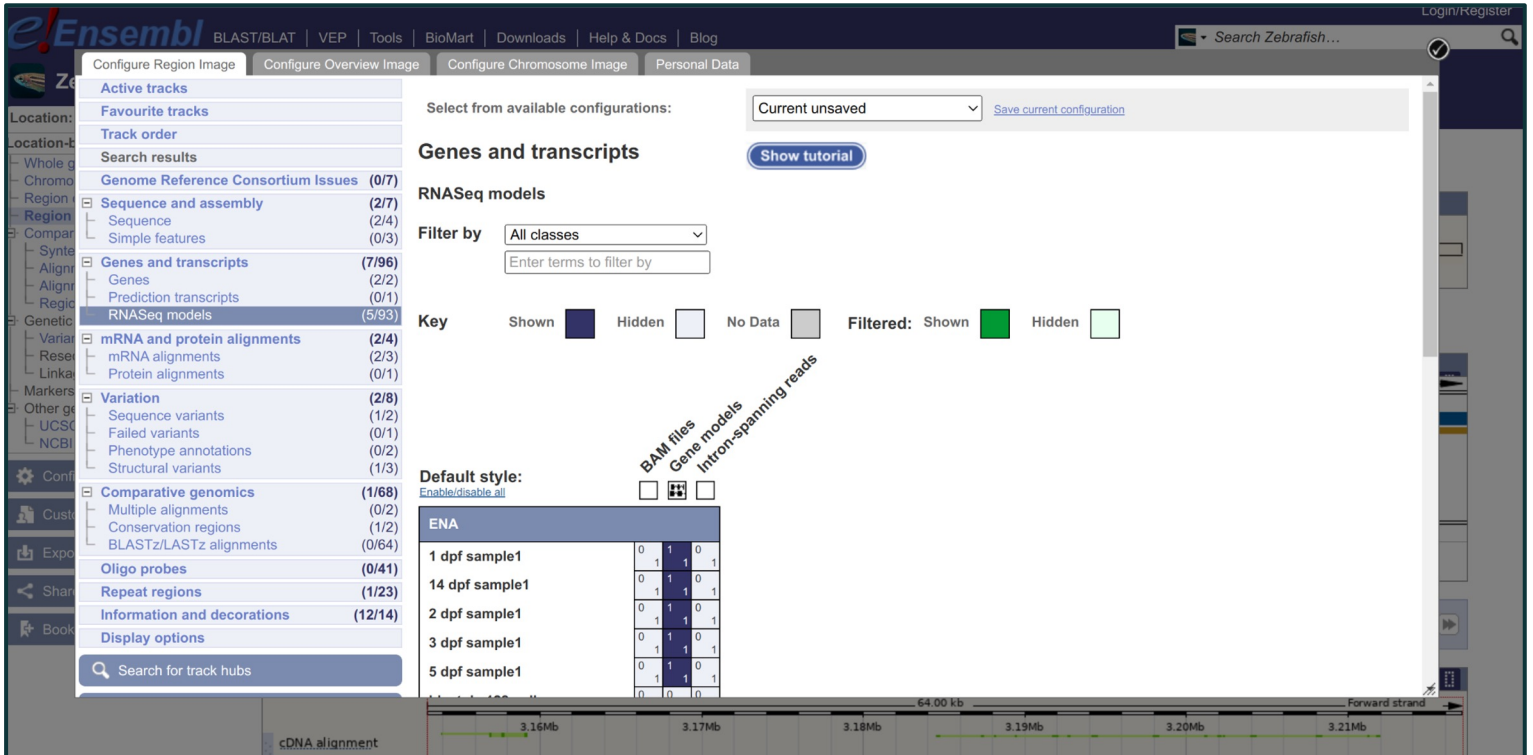
Part 2

- Configuring Ensembl tracks
- Ensembl “Gene” view
- Comparative genomics

- But first, back to the region we were looking at before the exercises: "22:3153000-3217000"

"Configure this page" Demo

- Go to "22:3153000-3217000" and click “Configure this page”

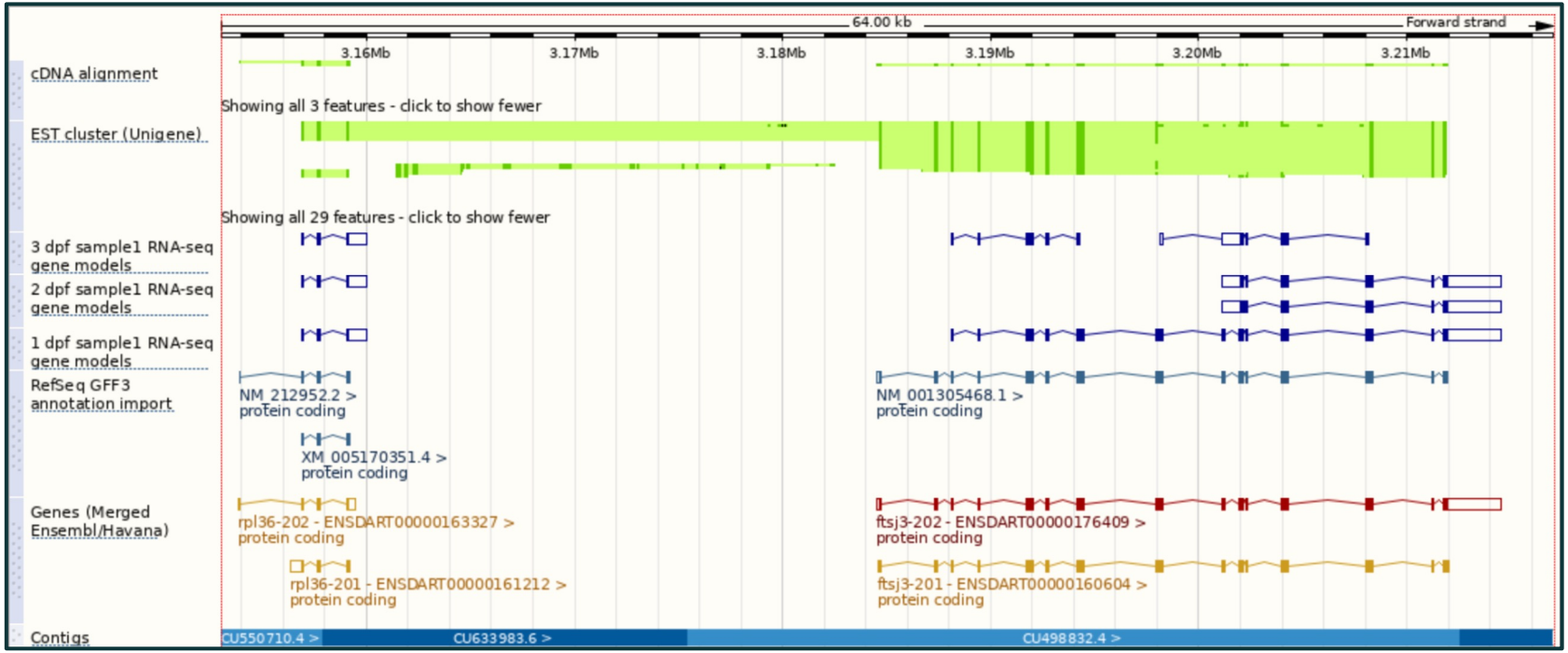


RefSeq Aside

- NCBI's **annotated** and **curated** database of reference sequences, including transcripts and proteins
- Accessions starting **X** are "Model RefSeq" **predictions** from automatic genome annotation
- Accessions starting **N** are "Known RefSeq" from **manually curated** cDNA and EST data
- Accessions starting **NM** & **XM** indicate mRNA; **NP** & **XP** are proteins

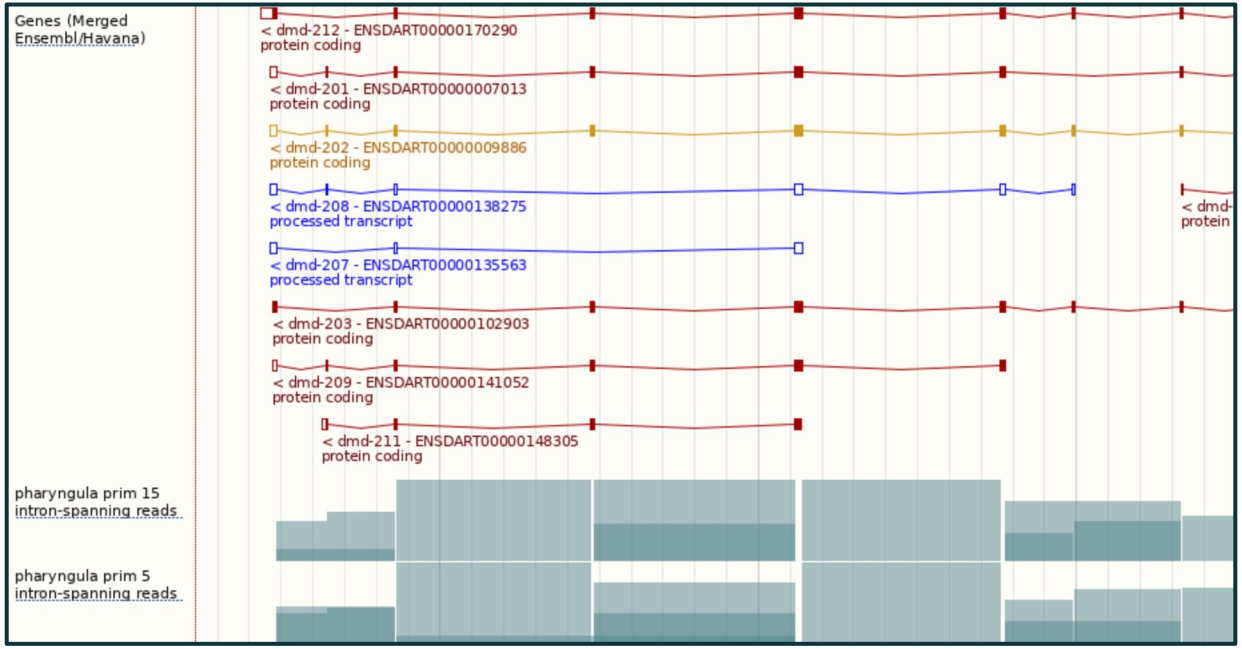
"Configure this page" Demo

- Go to "22:3153000-3217000" and click “Configure this page”



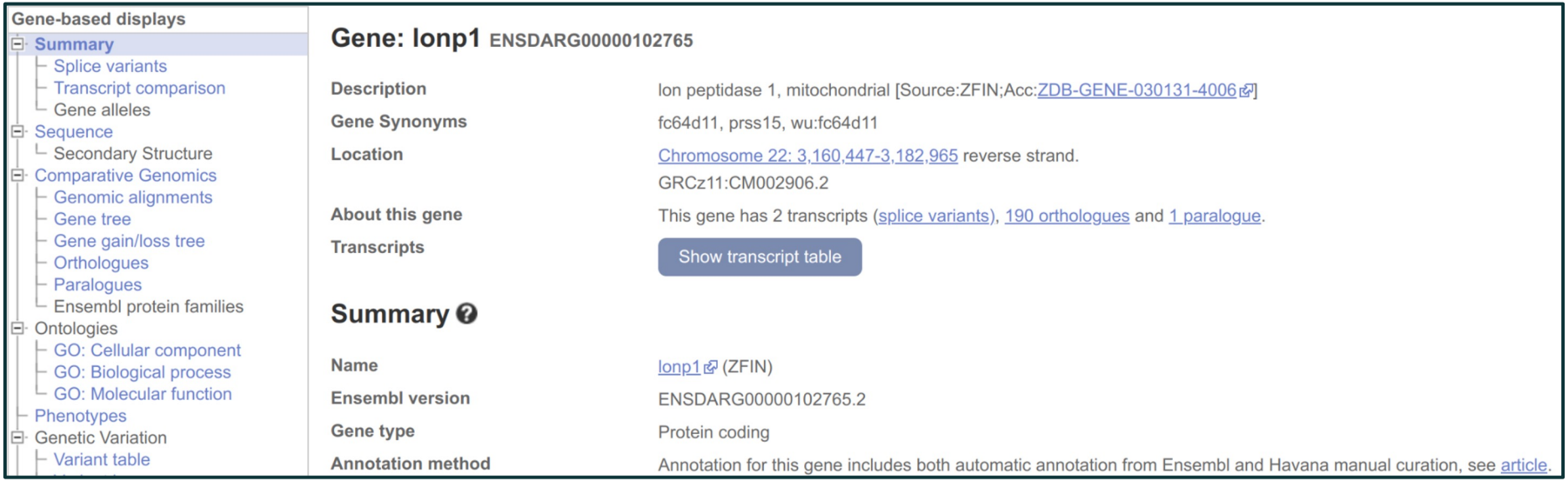
"Configure this page" Demo

- Go to "1:10822281-10882903" and click “Configure this page”
- Under “RNASeq models”, turn on “Intron-spanning reads” for “pharyngula prim 5” and “pharyngula prim 15”



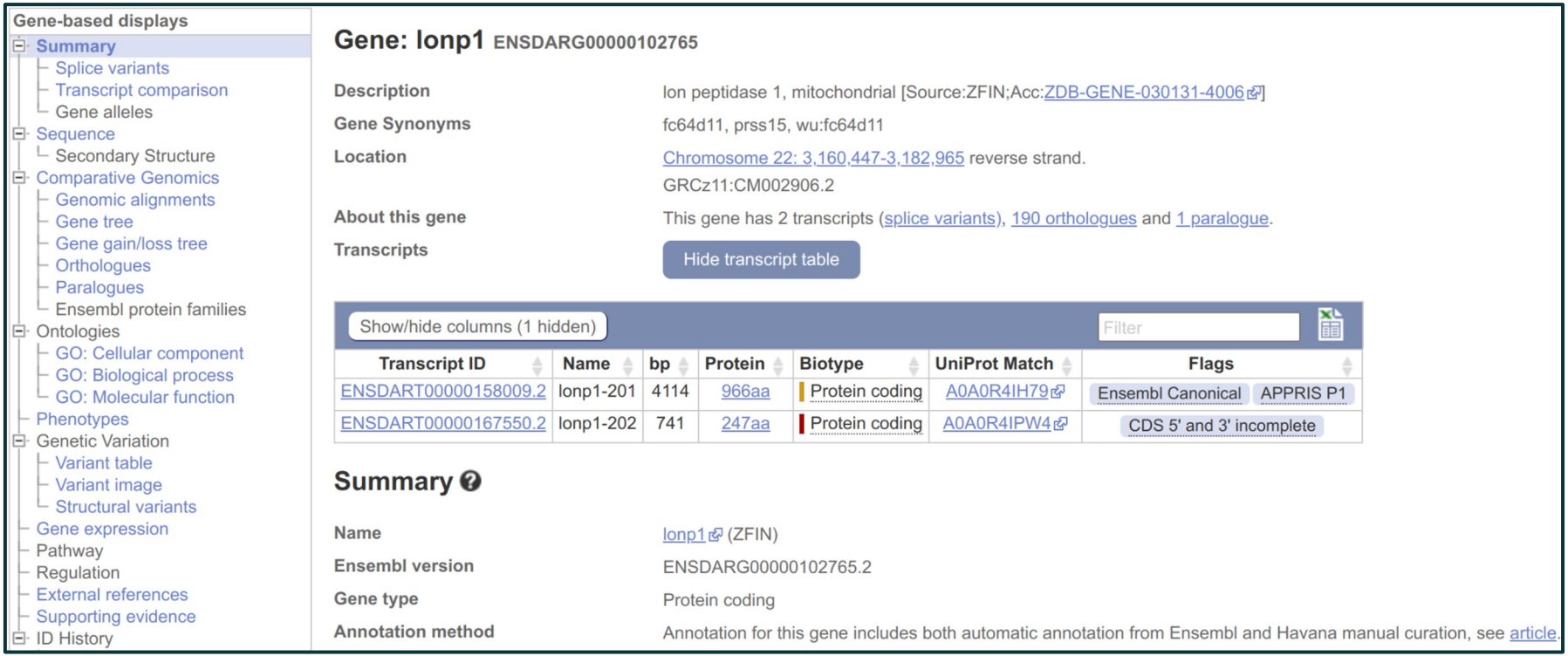
"Gene" Demo - Summary

- Go to ENSDARG00000102765



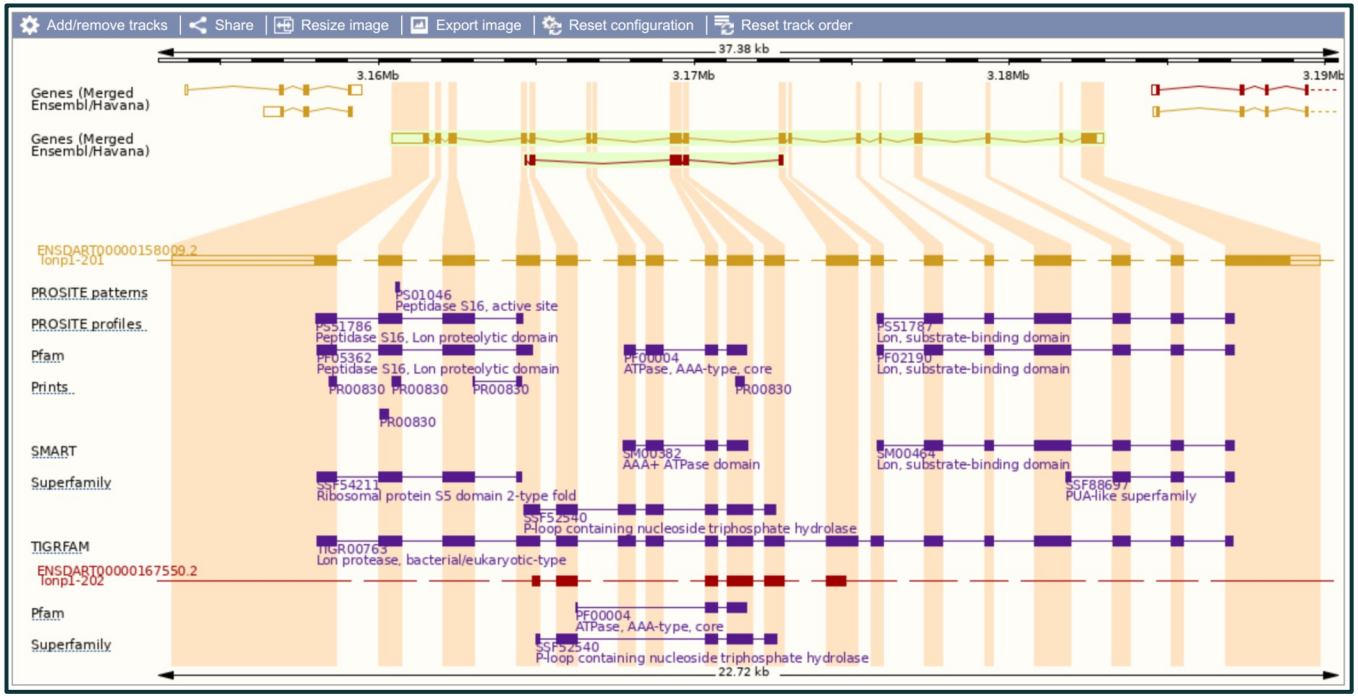
"Gene" Demo - Transcript Table

- Go to ENSDARG00000102765 and click on “Show transcript table”



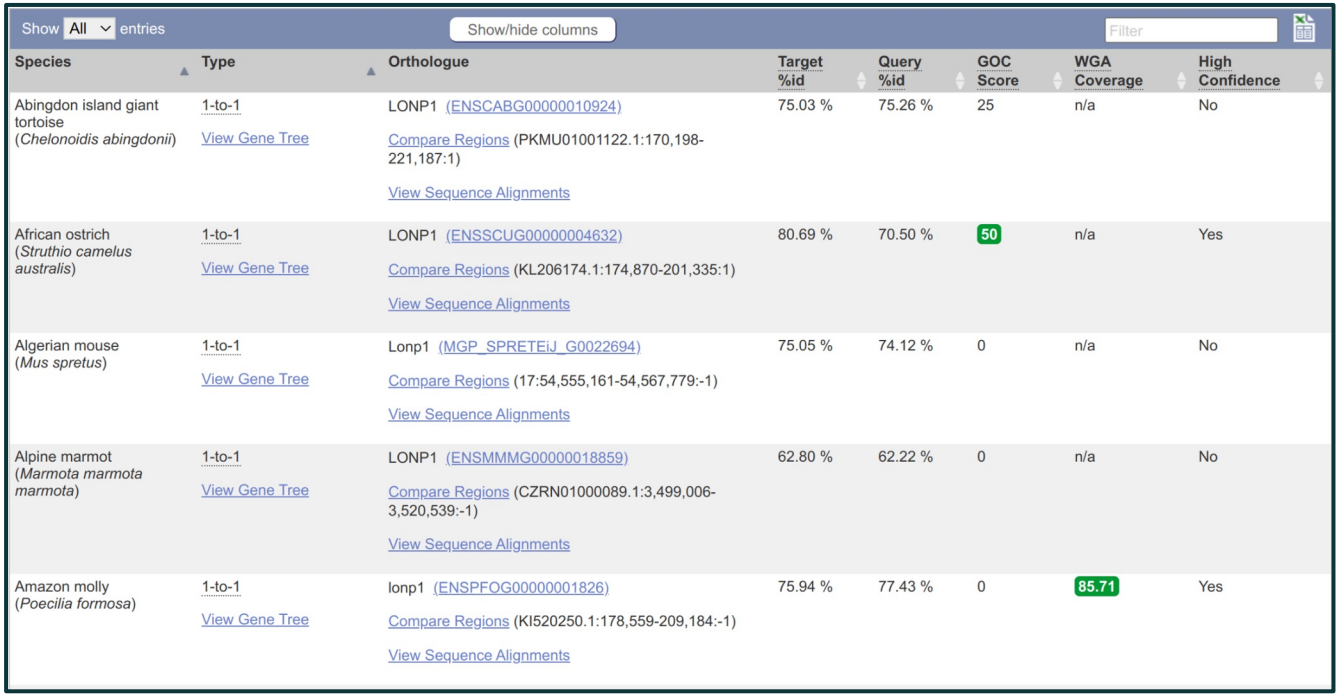
"Gene" Demo - Splice Variants

- Go to ENSDARG00000102765 and click on “Splice variants”



"Gene" Demo - Orthologues

- Go to ENSDARG00000102765 and click on “Orthologues”

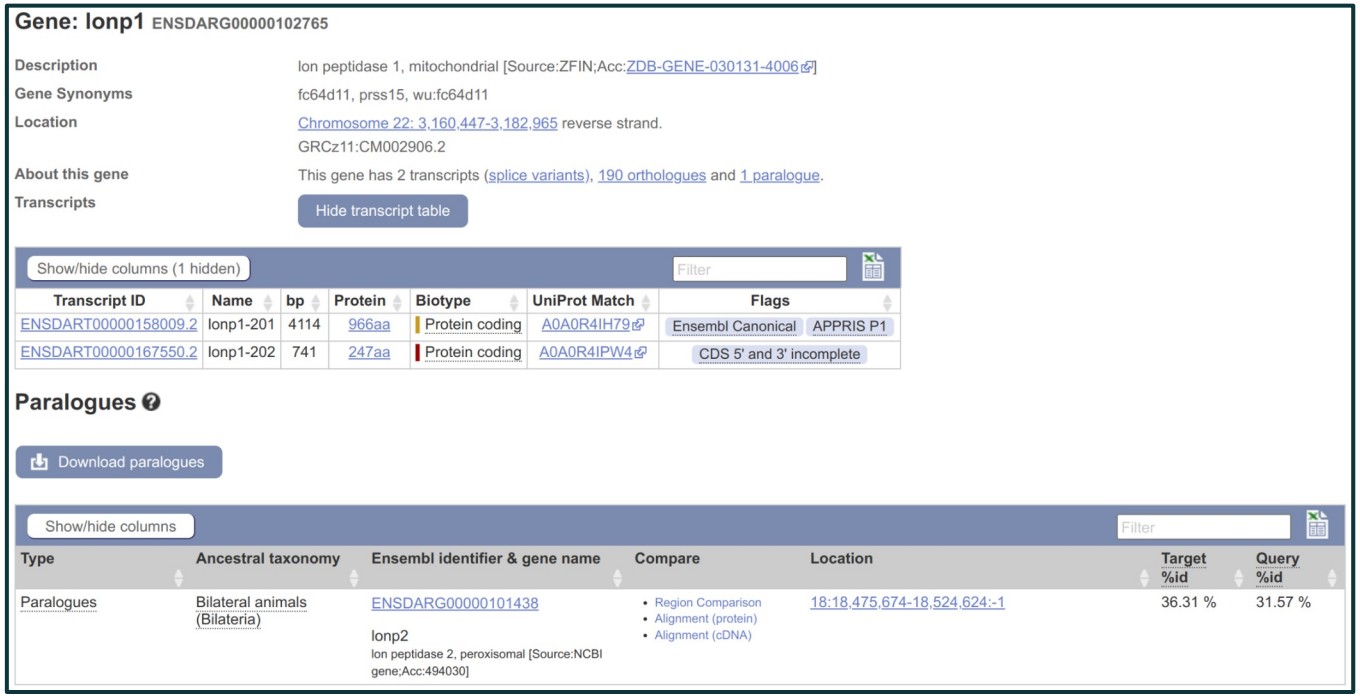


Show All entries | Show/hide columns | Filter

Species	Type	Orthologue	Target %id	Query %id	GOC Score	WGA Coverage	High Confidence
Abingdon island giant tortoise (*Chelonoidis abingdonii*)	1-to-1 View Gene Tree	LONP1 (ENSCABG00000010924) Compare Regions (PKMU01001122.1:170,198-221,187:1) View Sequence Alignments	75.03 %	75.26 %	25	n/a	No
African ostrich (*Struthio camelus australis*)	1-to-1 View Gene Tree	LONP1 (ENSSCUG00000004632) Compare Regions (KL206174.1:174,870-201,335:1) View Sequence Alignments	80.69 %	70.50 %	50	n/a	Yes
Algerian mouse (*Mus spretus*)	1-to-1 View Gene Tree	Lonp1 (MGP_SPRETEiJ_G0022694) Compare Regions (17:54,555,161-54,567,779:-1) View Sequence Alignments	75.05 %	74.12 %	0	n/a	No
Alpine marmot (*Marmota marmota marmota*)	1-to-1 View Gene Tree	LONP1 (ENSMMMG00000018859) Compare Regions (CZRN01000089.1:3,499,006-3,520,539:-1) View Sequence Alignments	62.80 %	62.22 %	0	n/a	No
Amazon molly (*Poecilia formosa*)	1-to-1 View Gene Tree	lonp1 (ENSPFOG00000001826) Compare Regions (KI520250.1:178,559-209,184:-1) View Sequence Alignments	75.94 %	77.43 %	0	85.71	Yes

"Gene" Demo - Paralogues

- Go to ENSDARG00000102765 and click on “Paralogues”



Gene: lonp1 ENSDARG00000102765

Description	lon peptidase 1, mitochondrial [Source:ZFIN;Acc:ZDB-GENE-030131-4006]
Gene Synonyms	fc64d11, prss15, wu:fc64d11
Location	Chromosome 22: 3,160,447-3,182,965 reverse strand. GRCz11:CM002906.2
About this gene	This gene has 2 transcripts (splice variants), 190 orthologues and 1 paralogue.
Transcripts	Hide transcript table

Show/hide columns (1 hidden) — Filter

Transcript ID	Name	bp	Protein	Biotype	UniProt Match	Flags
ENSDART00000158009.2	lonp1-201	4114	966aa	Protein coding	A0A0R4IH79	Ensembl Canonical, APPRIS P1
ENSDART00000167550.2	lonp1-202	741	247aa	Protein coding	A0A0R4IPW4	CDS 5' and 3' incomplete

Paralogues

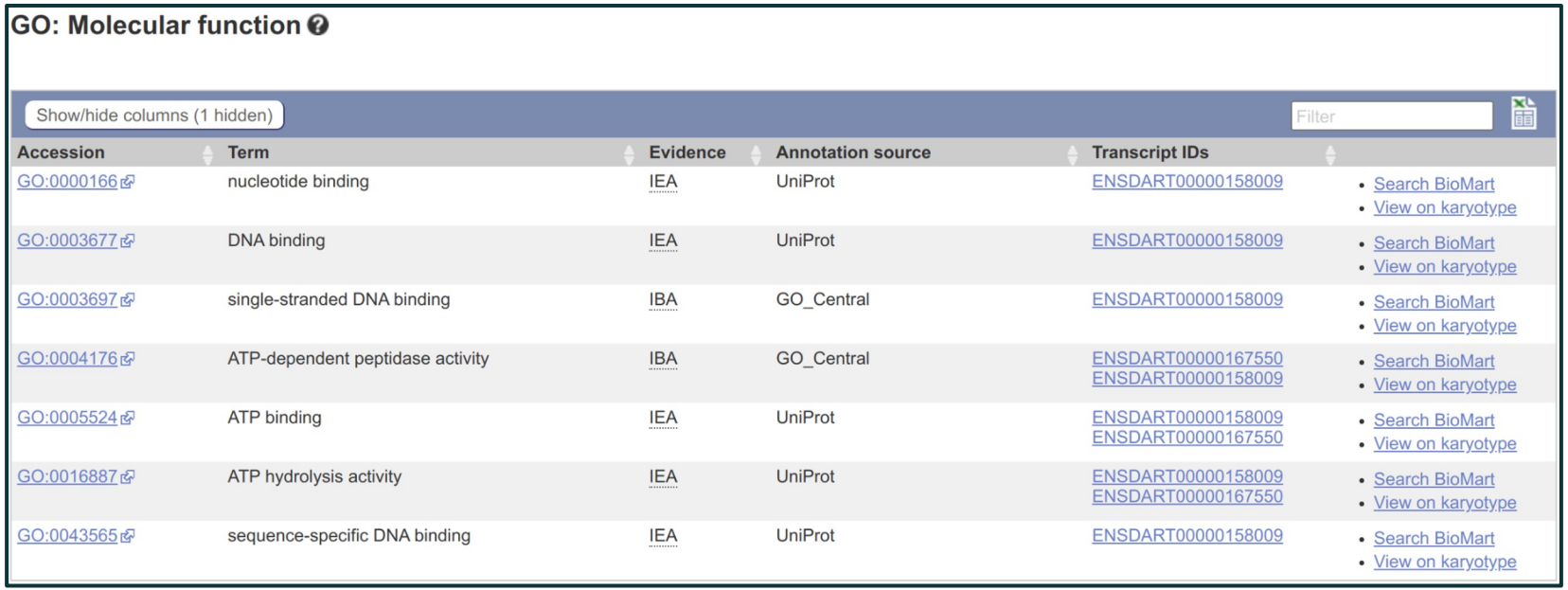
Download paralogues

Show/hide columns — Filter

Type	Ancestral taxonomy	Ensembl identifier & gene name	Compare	Location	Target %id	Query %id
Paralogues	Bilateral animals (Bilateria)	ENSDARG00000101438 lonp2 lon peptidase 2, peroxisomal [Source:NCBI gene;Acc:494030]	• Region Comparison • Alignment (protein) • Alignment (cDNA)	18:18,475,674-18,524,624:-1	36.31 %	31.57 %

"Gene" Demo - GO Terms

- Go to ENSDARG00000102765 and click on “GO: Molecular function”



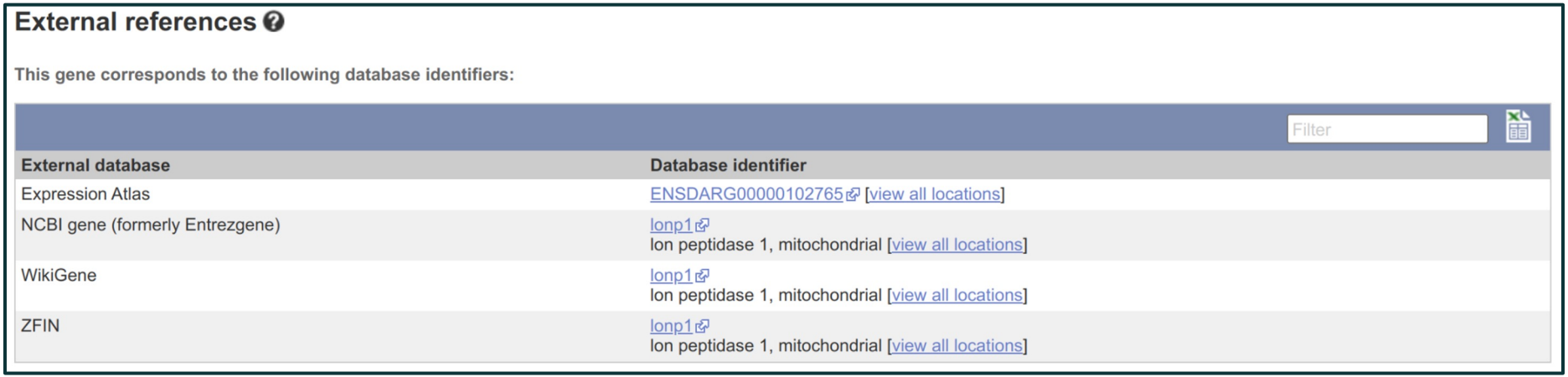
GO: Molecular function

Show/hide columns (1 hidden) Filter

Accession	Term	Evidence	Annotation source	Transcript IDs	
GO:0000166	nucleotide binding	IEA	UniProt	ENSDART00000158009	• Search BioMart • View on karyotype
GO:0003677	DNA binding	IEA	UniProt	ENSDART00000158009	• Search BioMart • View on karyotype
GO:0003697	single-stranded DNA binding	IBA	GO_Central	ENSDART00000158009	• Search BioMart • View on karyotype
GO:0004176	ATP-dependent peptidase activity	IBA	GO_Central	ENSDART00000167550 ENSDART00000158009	• Search BioMart • View on karyotype
GO:0005524	ATP binding	IEA	UniProt	ENSDART00000158009 ENSDART00000167550	• Search BioMart • View on karyotype
GO:0016887	ATP hydrolysis activity	IEA	UniProt	ENSDART00000158009 ENSDART00000167550	• Search BioMart • View on karyotype
GO:0043565	sequence-specific DNA binding	IEA	UniProt	ENSDART00000158009	• Search BioMart • View on karyotype

"Gene" Demo - External References

- Go to ENSDARG00000102765 and click on “External references”



External references

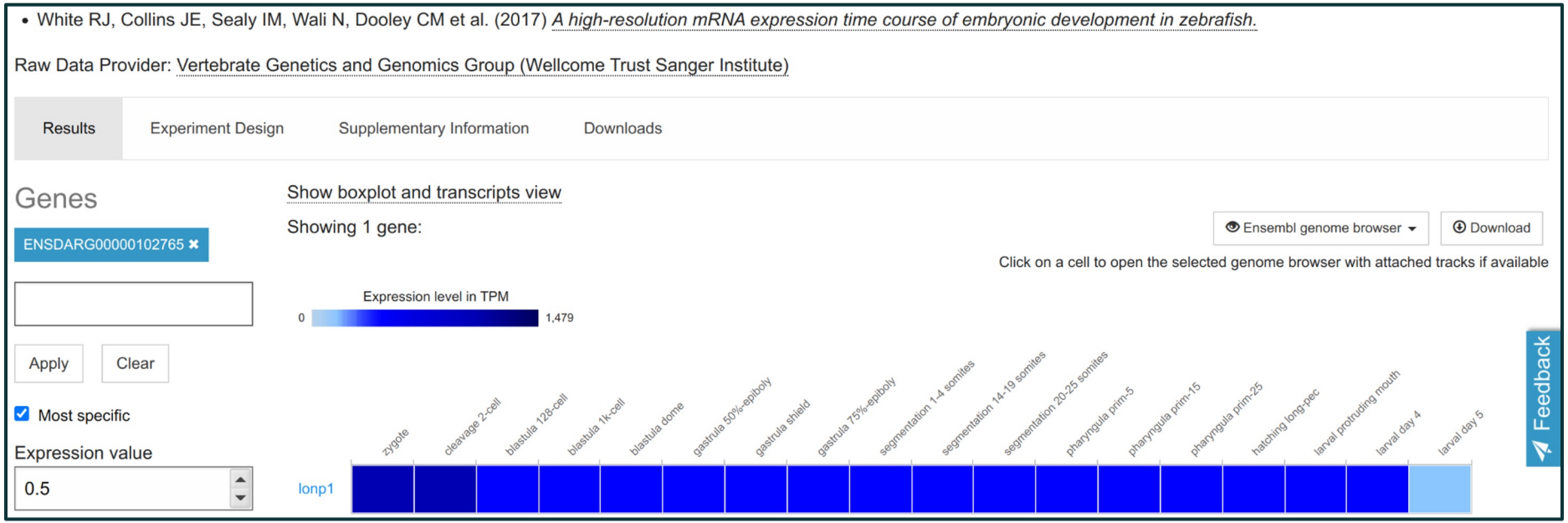
This gene corresponds to the following database identifiers:

Filter

External database	Database identifier
Expression Atlas	ENSDARG00000102765 [view all locations]
NCBI gene (formerly Entrezgene)	lonp1 lon peptidase 1, mitochondrial [view all locations]
WikiGene	lonp1 lon peptidase 1, mitochondrial [view all locations]
ZFIN	lonp1 lon peptidase 1, mitochondrial [view all locations]

"Gene" Demo - Expression Atlas

- From “External references” click “Expression Atlas” ID then “18 White et al”

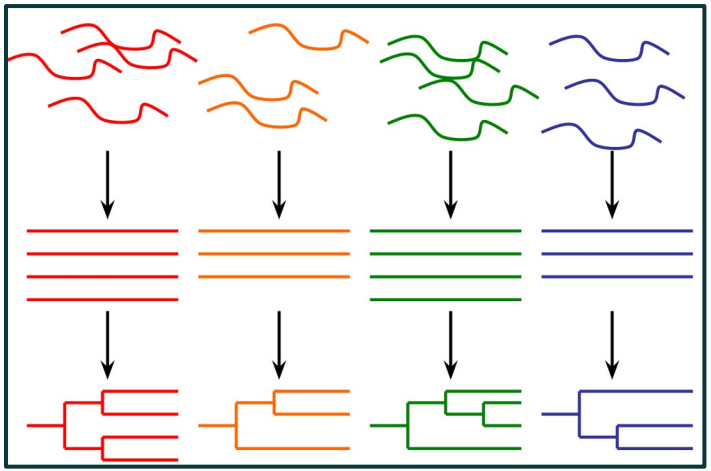


Compara

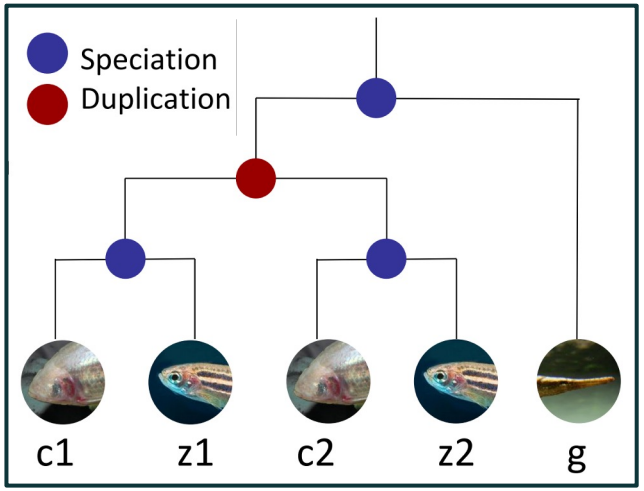
- Compara - produce Ensembl's comparative genomics resources
- Two types of analysis:
  - Gene level comparisons to produce **gene trees**, e.g. infer **homologues** (orthologues & paralogues)
  - **Whole genome alignments** - pairwise and multiple alignments, e.g. **constrained elements** and **synteny**

Compara - Gene Trees

- Separate trees for **proteins** and **ncRNAs** (take secondary structure into account)
- Process:
  - Take **representative** transcripts (e.g. longest CDS) from all genes from all species
  - Classify genes into **clusters** by TreeFam family
  - Build **multiple** alignment
  - Build **gene tree** reconciled with NCBI's taxonomy tree
  - Infer **orthologues** and **paralogues**



Compara - Infer Homologues (Orthologues & Paralogues)



z1 & z2 are **paralogues** (arose from **duplication**), as are **c1 & c2**

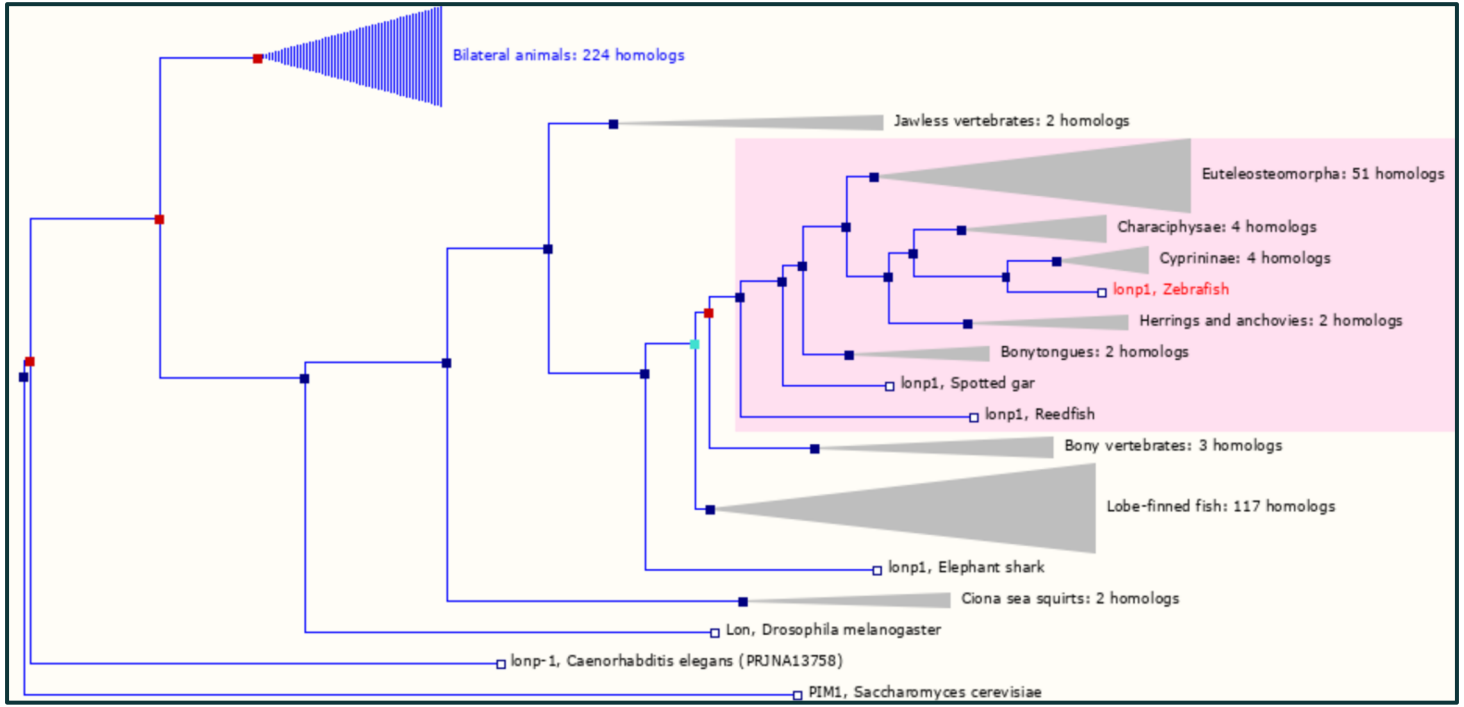
z1 & c1 are **orthologues** (arose from **speciation**), as are **z2 & c2** + **z2 & g**, etc…

z1 & c1 have a **one-to-one** relationship

g has a **one-to-many** relationship to e.g. **z1** and **z2**

Homologues labelled “**high confidence**” are supported by conservation of synteny or whole genome alignment blocks

Compara - lonp1 Gene Tree

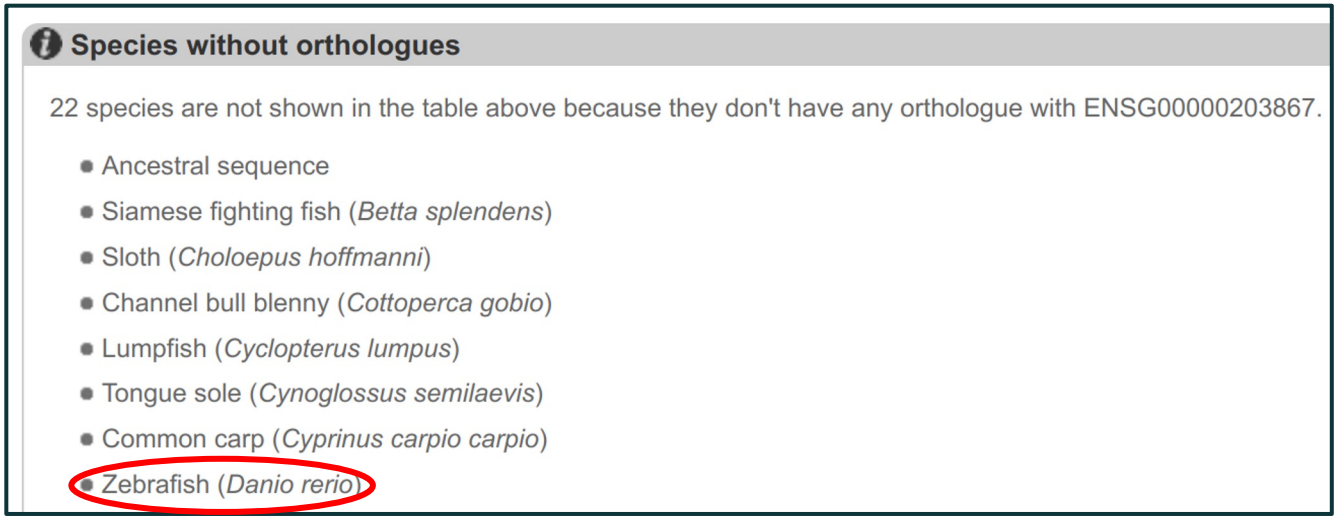


Compara - Whole Genome Alignments

- **Pairwise whole genome alignments** with LASTZ
- Zebrafish has alignments to **64 species** (plus itself)
- Only human (181) and medaka (65) have more
- Full list at: www.ensembl.org/info/genome/compara/analyses.html
- **Multiple genome alignments** with EPO (Enredo, Pecan, Ortheus)
- Zebrafish is in **2** alignments (out of 11 in Ensembl) - one of **39 fish** and one of **65 fish**
- For lists of species, see: www.ensembl.org/info/genome/compara/multiple_genome_alignments.html

Synteny Example

- No zebrafish orthologue listed for human RBM20 gene (ENSG00000203867)



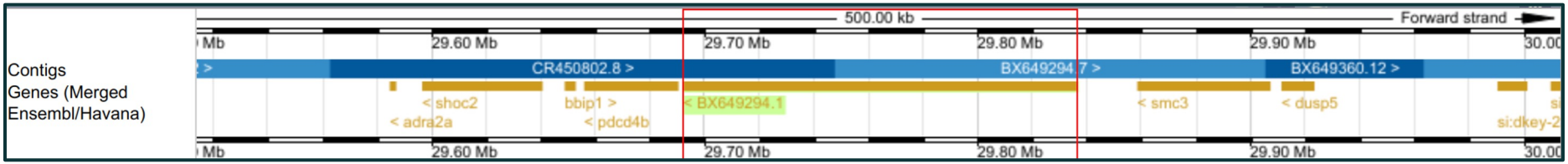
Synteny Example

- If we look at the region around RBM20 in human and then click on **Synteny** we see conservation of synteny with zebrafish chr22

Homo sapiens genes	Location		*Danio rerio* homologues	Location	
DUSP5 (ENSG00000138166)	10:110497907-110511533	→	dusp5 (ENSDARG00000019307)	22:29911326-29922872	Region Comparison
SMC3 (ENSG00000108055)	10:110567684-110606048	→	smc3 (ENSDARG00000019000)	22:29858535-29906764	Region Comparison
RBM20 (ENSG00000203867)	10:110644336-110839468		No homologues		
PDCD4 (ENSG00000150593)	10:110871795-110900006	→	pdcd4b (ENSDARG00000041022)	22:29655981-29689981	Region Comparison
BBIP1 (ENSG00000214413)	10:110898730-110919201	→	bbip1 (ENSDARG00000071046)	22:29648854-29652356	Region Comparison
SHOC2 (ENSG00000108061)	10:110919367-111017307	→	shoc2 (ENSDARG00000040853)	22:29596646-29640181	Region Comparison
ADRA2A (ENSG00000150594)	10:111077029-111080907	→	adra2a (ENSDARG00000040841)	22:29584800-29586608	Region Comparison

Synteny Example

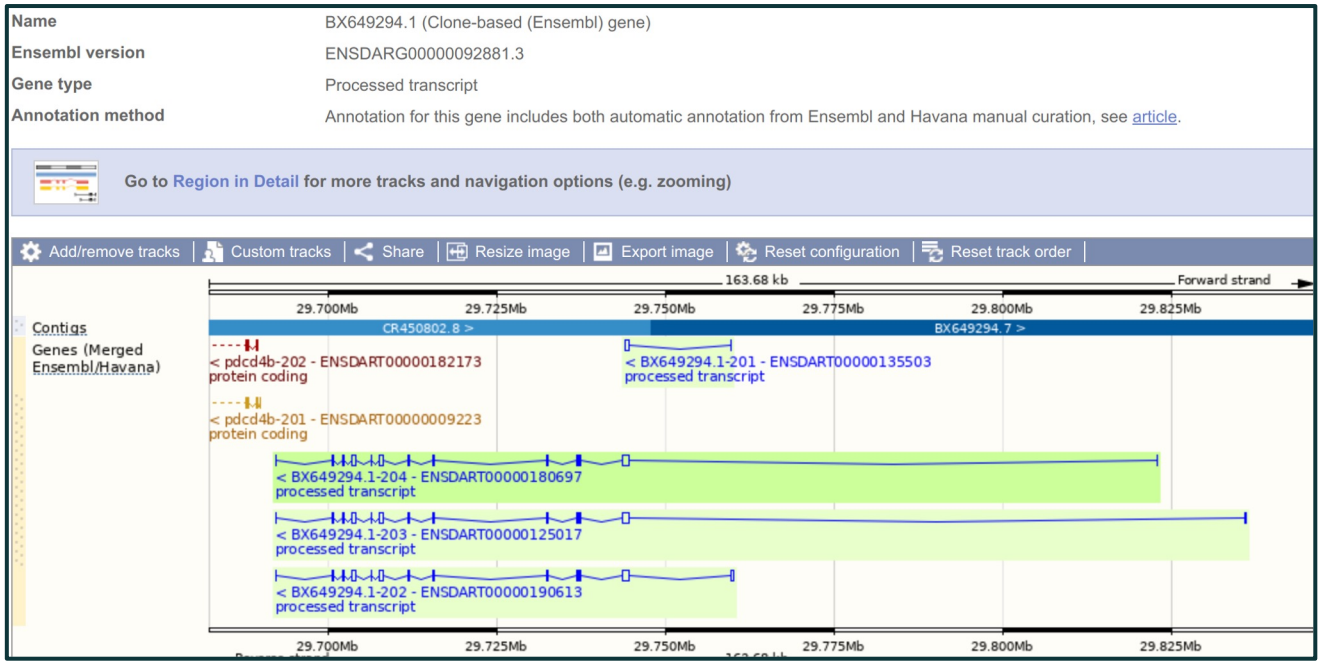
- If we look at the chr22 region in zebrafish then all the surrounding genes are the same and RBM20 is likely to be BX649294.1



Homo sapiens genes	Location		*Danio rerio* homologues	Location	
DUSP5 (ENSG00000138166)	10:110497907-110511533	→	dusp5 (ENSDARG00000019307)	22:29911326-29922872	Region Comparison
SMC3 (ENSG00000108055)	10:110567684-110606048	→	smc3 (ENSDARG00000019000)	22:29858535-29906764	Region Comparison
RBM20 (ENSG00000203867)	10:110644336-110839468		No homologues		
PDCD4 (ENSG00000150593)	10:110871795-110900006	→	pdcd4b (ENSDARG00000041022)	22:29655981-29689981	Region Comparison
BBIP1 (ENSG00000214413)	10:110898730-110919201	→	bbip1 (ENSDARG00000071046)	22:29648854-29652356	Region Comparison
SHOC2 (ENSG00000108061)	10:110919367-111017307	→	shoc2 (ENSDARG00000040853)	22:29596646-29640181	Region Comparison
ADRA2A (ENSG00000150594)	10:111077029-111080907	→	adra2a (ENSDARG00000040841)	22:29584800-29586608	Region Comparison

Synteny Example

- Erroneously labelled as processed transcript and so not in protein gene tree, so not labelled as orthologue or named by orthology



Exercise 2

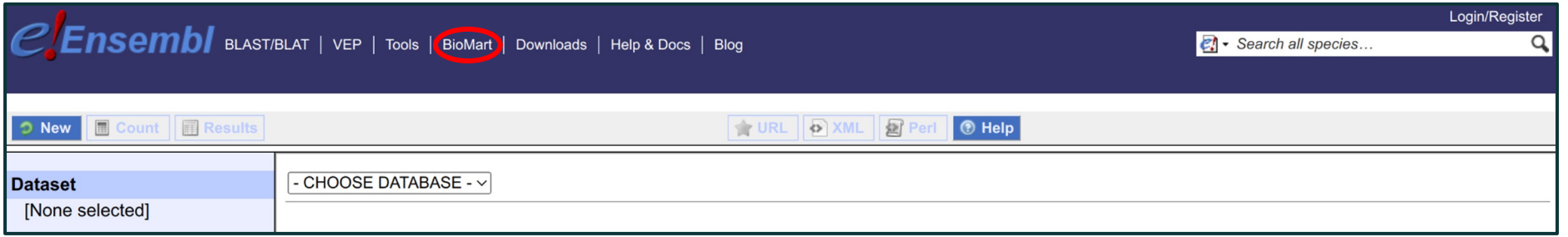
- Do Exercise 2 - “exploring genes”
- Covers:
  - Gene view
  - Phenotypes
  - Gene Ontology
  - Homologues
  - Gene trees
  - Synteny
- Go to mbl2023.buschlab.org

Part 3

- BioMart
- Other tools
- Custom tracks

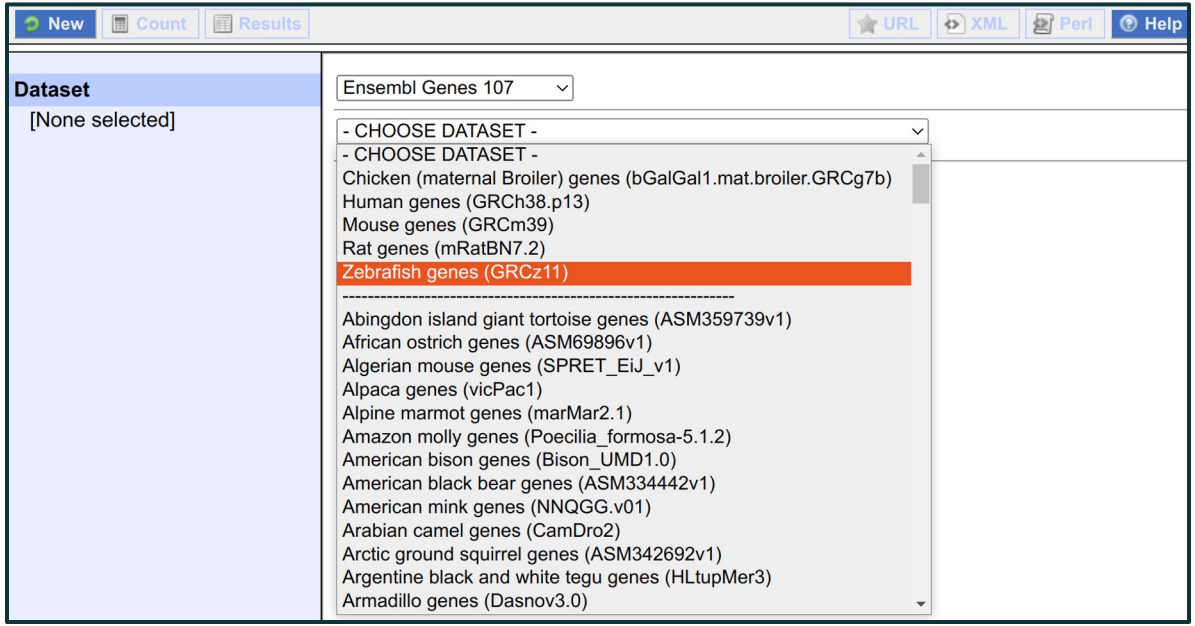
BioMart

- **Export** (large amounts of) Ensembl data without programming
- Completely **customisable**, but **simple** to make complex queries
- Four stages:
  - Dataset
  - Filters
  - Attributes
  - Results



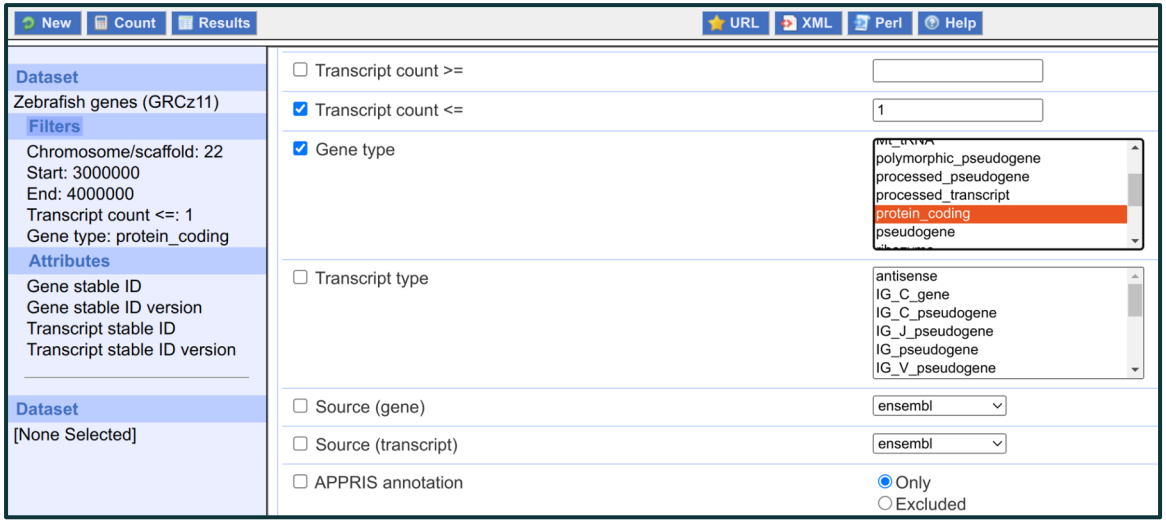
BioMart - Dataset

- Choose **database** (e.g. genes or variants) and **species**



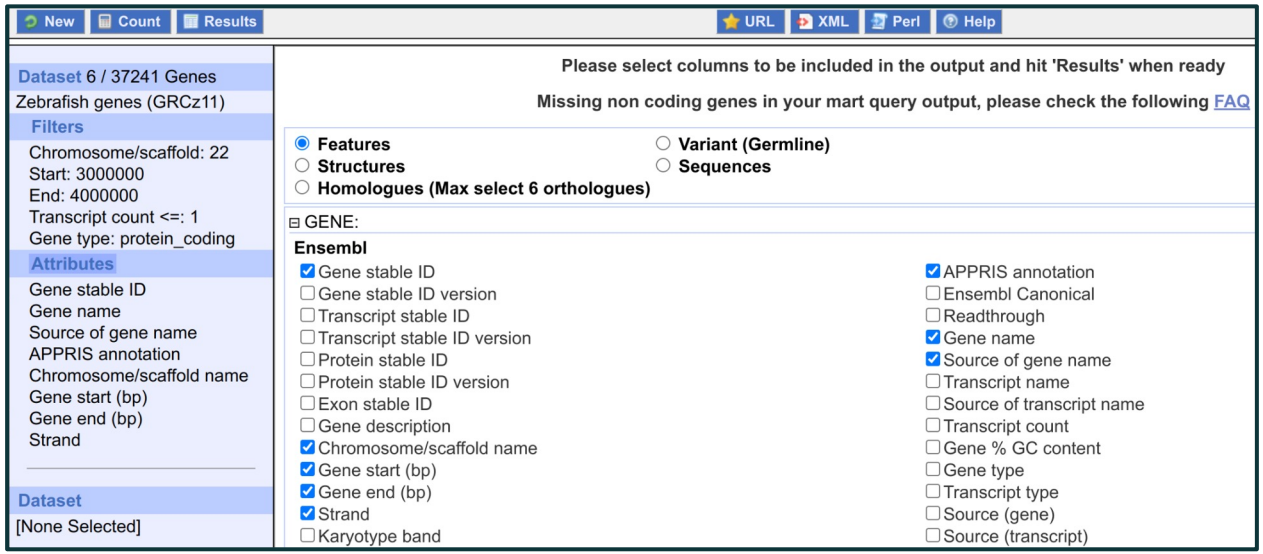
BioMart - Filters

- **Filter** to reduce the dataset
- Can select **multiple** filters
- e.g. regions, IDs, GO terms, etc...



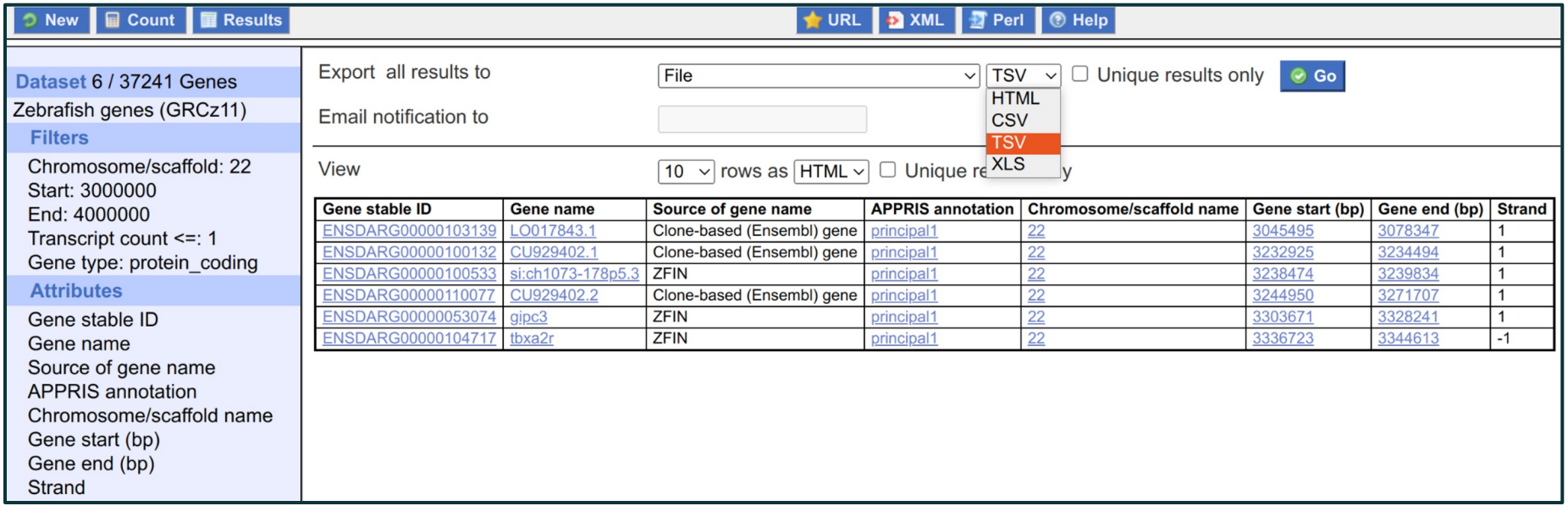
BioMart - Attributes

- What data to **export**
- e.g. IDs, genomic locations, sequences, homologues, etc...

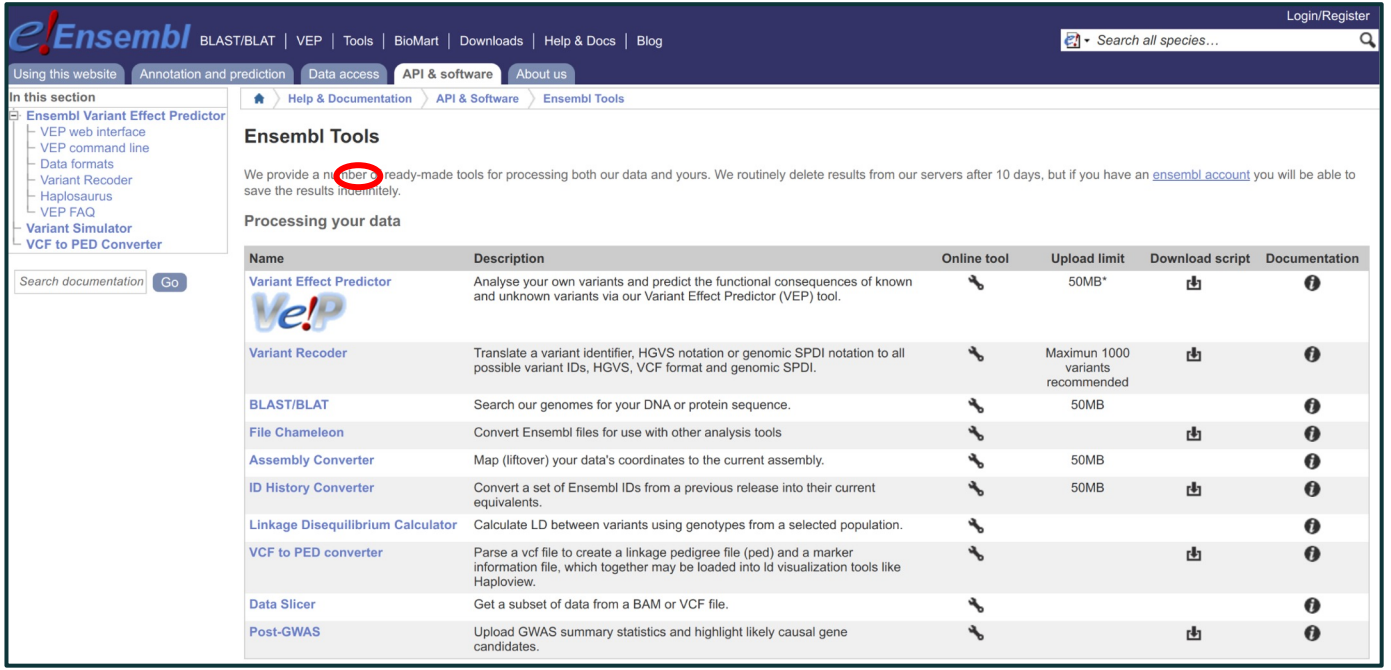


BioMart - Results

- Access your selected data in multiple formats
- e.g. HTML, TSV, CSV, XLS



More Tools



- Results from all tools can be stored indefinitely if create an **Ensembl account**

Variant Effect Predictor

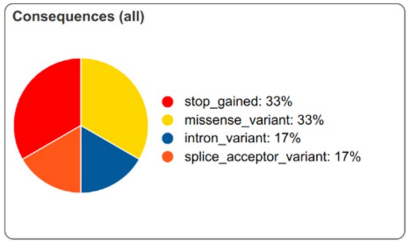
- VEP predicts **consequences** of variants
- www.ensembl.org/Danio_rerio/Tools/VEP
- Example:

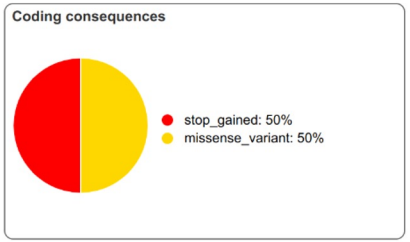
```
22 3169475 3169475 G/T 1
22 3169514 3169514 A/T 1
22 3166910 3166910 C/A 1
```

  (Chr, Start, End, REF/ALT, Strand)
- Custom Ensembl format, but standard formats like **VCF** can be used

Variant Effect Predictor

Category	Count
Variants processed	3
Variants filtered out	0
Novel / existing variants	-
Overlapped genes	1
Overlapped transcripts	2
Overlapped regulatory features	-





Results preview

Navigation (per variant) — Page: 1 of 1 | Show: 1 All variants

Filters — Uploaded variant | is | defined | Add

Download — All: VCF VEP TXT — BioMart: Variants Genes

New job

Show/hide columns (16 hidden)

Uploaded variant	Location	Allele	Consequence	Symbol	Gene	Feature	Biotype	cDNA position	CDS position	Protein position	Amino acids	Codons	APPRIS	SIFT
22_3166910_C/A	22:3166910-3166910	A	splice_acceptor_variant	lonp1	ENSDARG00000102765	ENSDART00000158009.2	protein_coding	-	-	-	-	-	P1	-
22_3166910_C/A	22:3166910-3166910	A	intron_variant	lonp1	ENSDARG00000102765	ENSDART00000167550.2	protein_coding	-	-	-	-	-	-	-
22_3169475_G/T	22:3169475-3169475	T	stop_gained	lonp1	ENSDARG00000102765	ENSDART00000158009.2	protein_coding	1885	1674	558	Y/*	TAC/TAA	P1	-
22_3169475_G/T	22:3169475-3169475	T	stop_gained	lonp1	ENSDARG00000102765	ENSDART00000167550.2	protein_coding	405	405	135	Y/*	TAC/TAA	-	-
22_3169514_A/T	22:3169514-3169514	T	missense_variant	lonp1	ENSDARG00000102765	ENSDART00000158009.2	protein_coding	1846	1635	545	S/R	AGT/AGA	P1	0
22_3169514_A/T	22:3169514-3169514	T	missense_variant	lonp1	ENSDARG00000102765	ENSDART00000167550.2	protein_coding	366	366	122	S/R	AGT/AGA	-	0

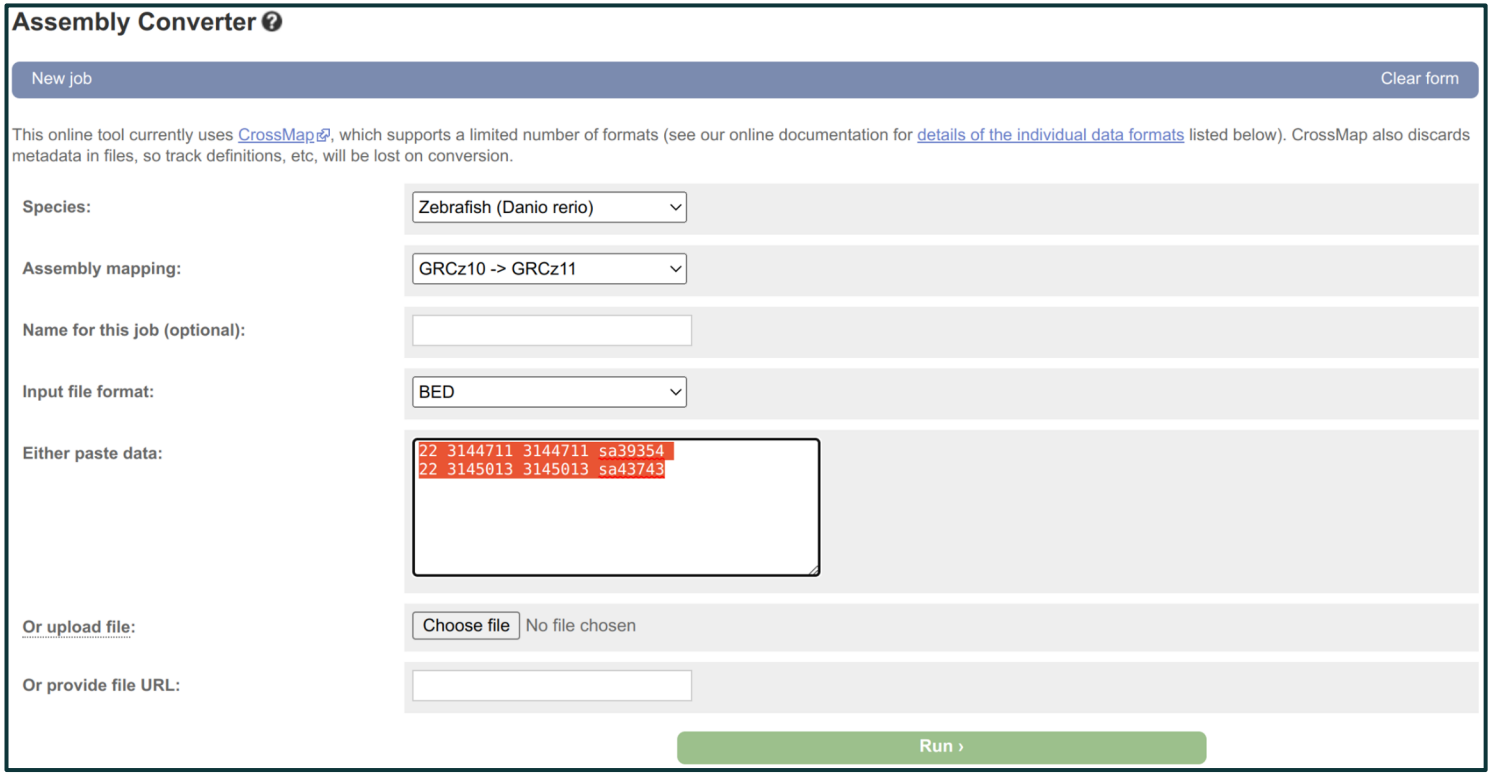
Assembly Converter

- Assembly Converter allows converting coordinates from one assembly to another
- Also known as **LiftOver**
- e.g. used for converting coordinates found in old papers
- www.ensembl.org/Danio_rerio/Tools/AssemblyConverter
- Example:

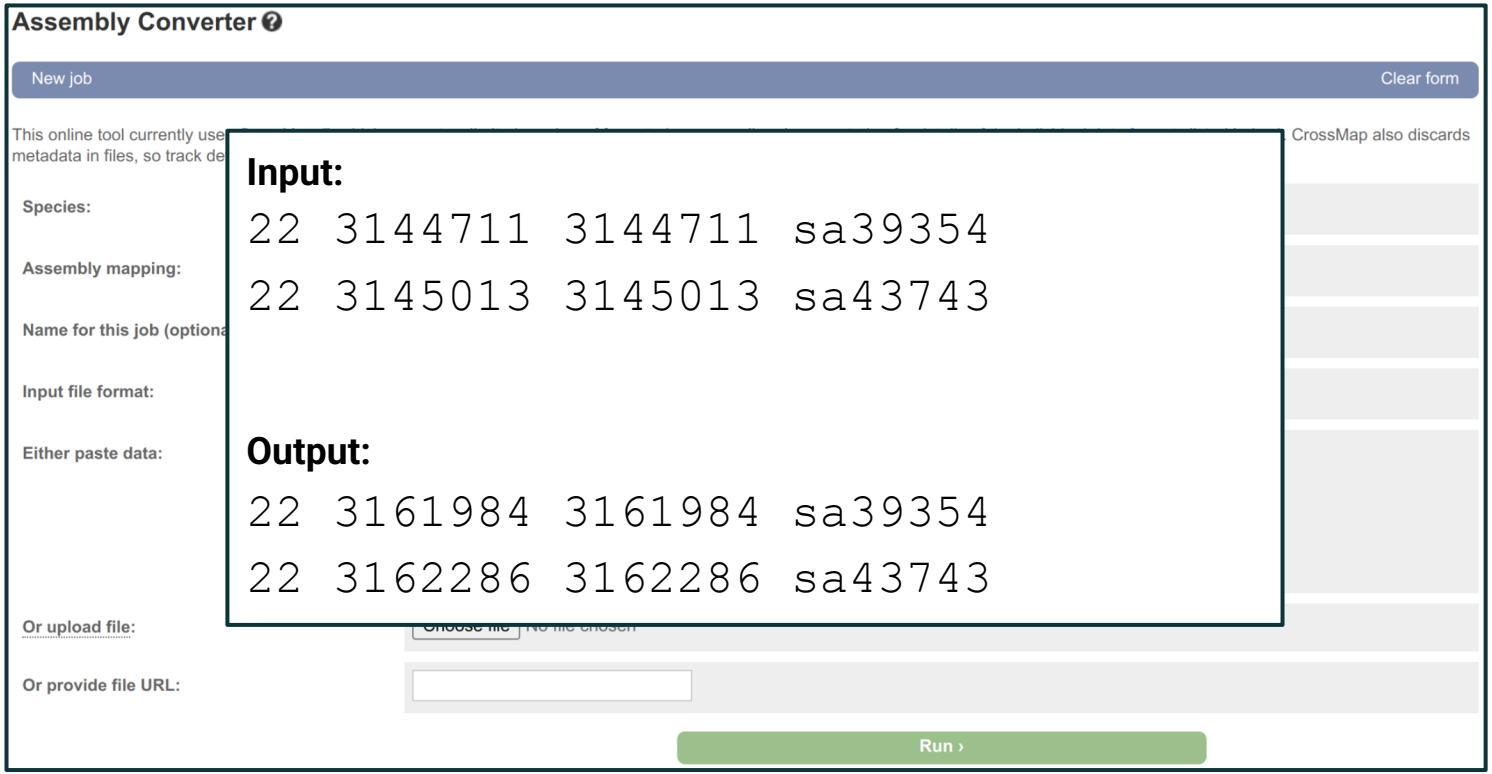
```
22 3144711 3144711 sa39354
22 3145013 3145013 sa43743
```

  (Chr, Start, End, Name)
- **BED format:** www.ensembl.org/info/website/upload/bed.html
- (Only first three fields are essential)

Assembly Converter



Assembly Converter

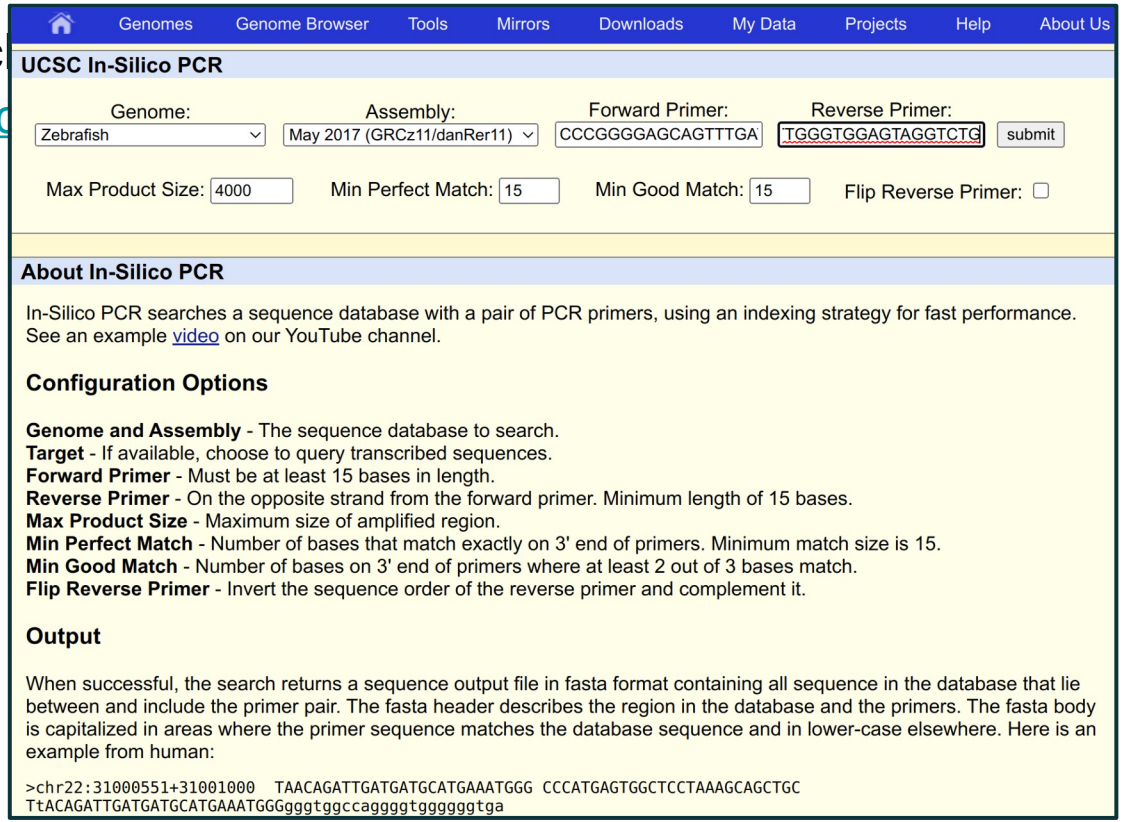


UCSC In-Silico PCR

- Fast search for possible products from a pair of **PCR** primers
- genome.ucsc.edu/cgi-bin/hgPcr

UCSC In-Silico PCR

- Fast searc
- genome.uc



Genomes | Genome Browser | Tools | Mirrors | Downloads | My Data | Projects | Help | About Us

UCSC In-Silico PCR

Genome: Zebrafish | Assembly: May 2017 (GRCz11/danRer11) | Forward Primer: CCCGGGGAGCAGTTTGA | Reverse Primer: TGGGTGGAGTAGGTCTG | submit

Max Product Size: 4000 | Min Perfect Match: 15 | Min Good Match: 15 | Flip Reverse Primer:

About In-Silico PCR

In-Silico PCR searches a sequence database with a pair of PCR primers, using an indexing strategy for fast performance. See an example video on our YouTube channel.

Configuration Options

Genome and Assembly - The sequence database to search.
Target - If available, choose to query transcribed sequences.
Forward Primer - Must be at least 15 bases in length.
Reverse Primer - On the opposite strand from the forward primer. Minimum length of 15 bases.
Max Product Size - Maximum size of amplified region.
Min Perfect Match - Number of bases that match exactly on 3' end of primers. Minimum match size is 15.
Min Good Match - Number of bases on 3' end of primers where at least 2 out of 3 bases match.
Flip Reverse Primer - Invert the sequence order of the reverse primer and complement it.

Output

When successful, the search returns a sequence output file in fasta format containing all sequence in the database that lie between and include the primer pair. The fasta header describes the region in the database and the primers. The fasta body is capitalized in areas where the primer sequence matches the database sequence and in lower-case elsewhere. Here is an example from human:

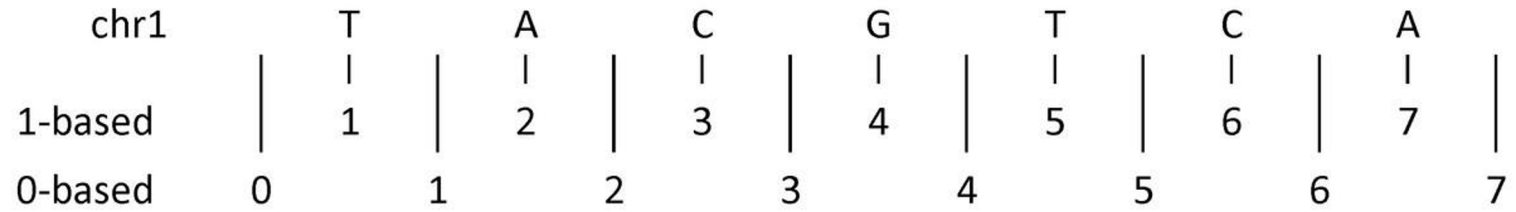
```
>chr22:31000551+31001000  TAACAGATTGATGATGCATGAAATGGG CCCATGAGTGGCTCCTAAAGCAGCTGC
TtACAGATTGATGATGCATGAAATGGGgggtggccaggggtgggggtga
```

UCSC In-Silico PCR

- Fast search for p mers
- genome.ucsc.edu

UCSC & Ensembl Differences

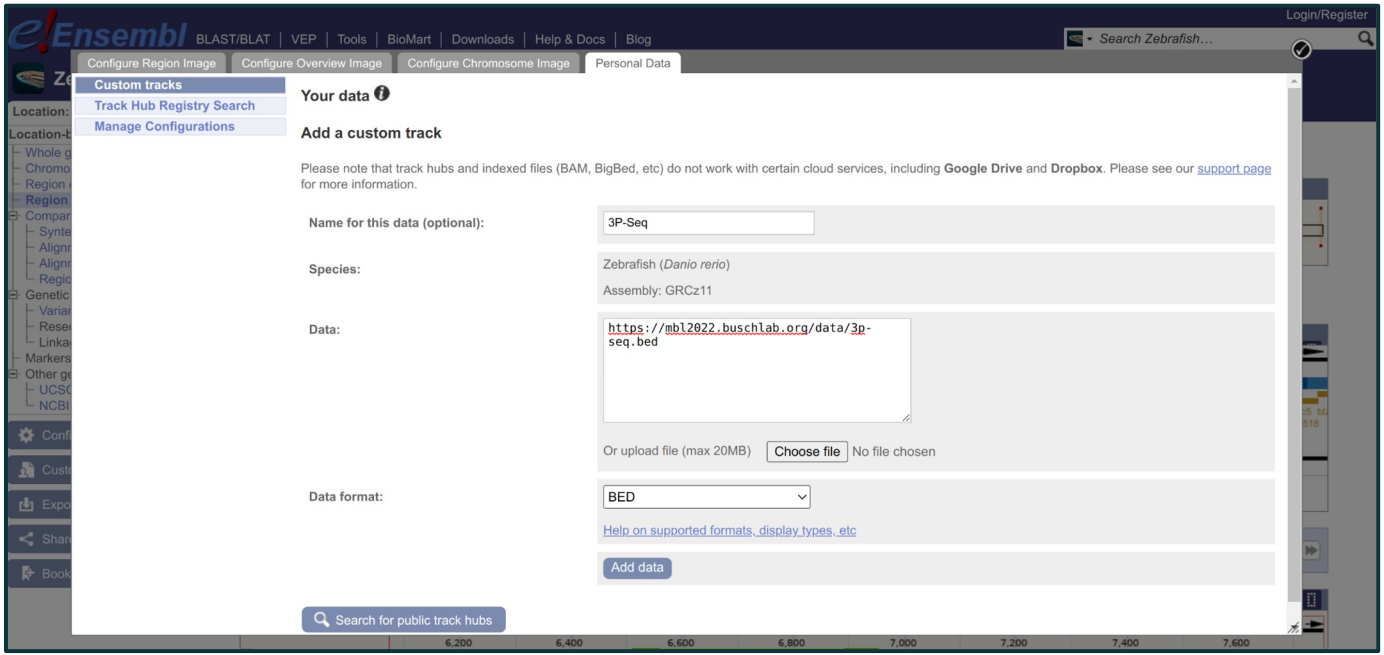
- **Ensembl:** 1
  UCSC: chr1
- **Ensembl:** 1-based coordinates (bases numbered)
  UCSC: 0-based coordinates (numbers between bases)



- The **G** is **1:4-4** in Ensembl coordinates but **1:3-4** in UCSC

Custom Tracks

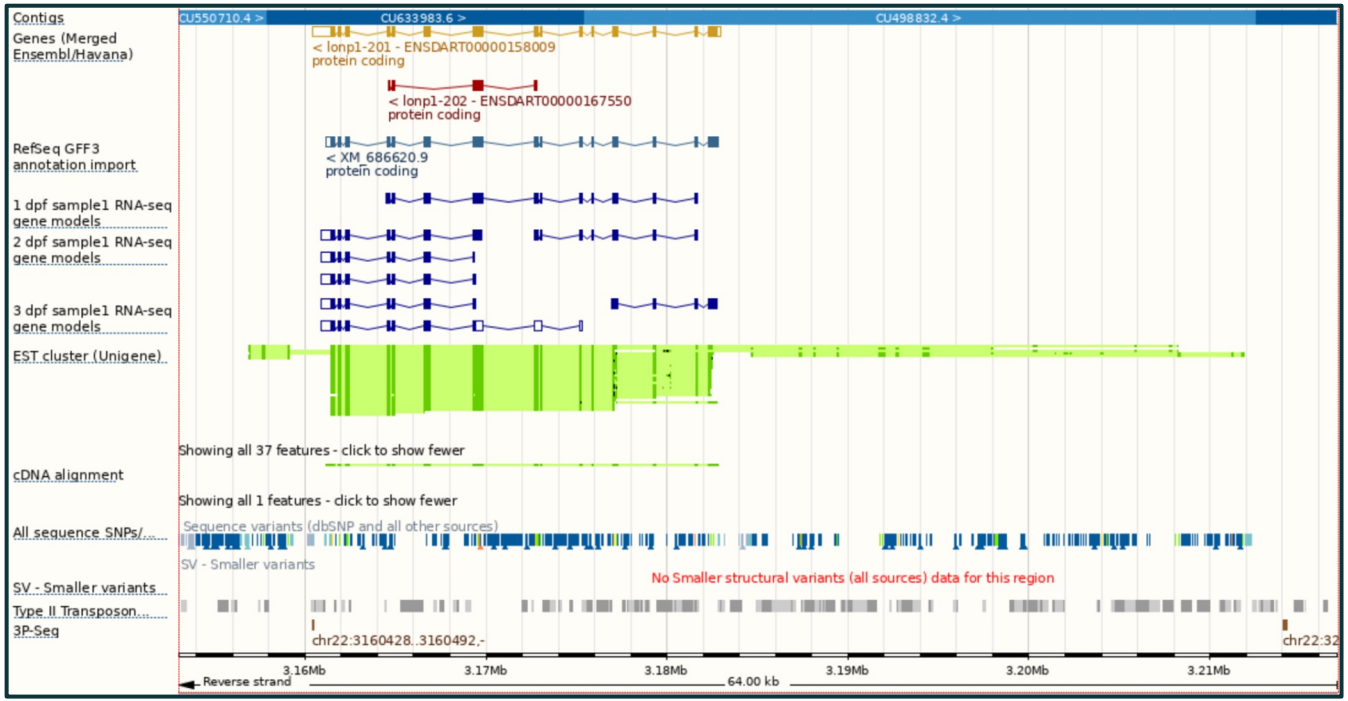
- Click “Custom tracks” and add https://mbl2023.buschlab.org/data/3p-seq.bed



- 24 hpf 3P-Seq data from Bartel lab

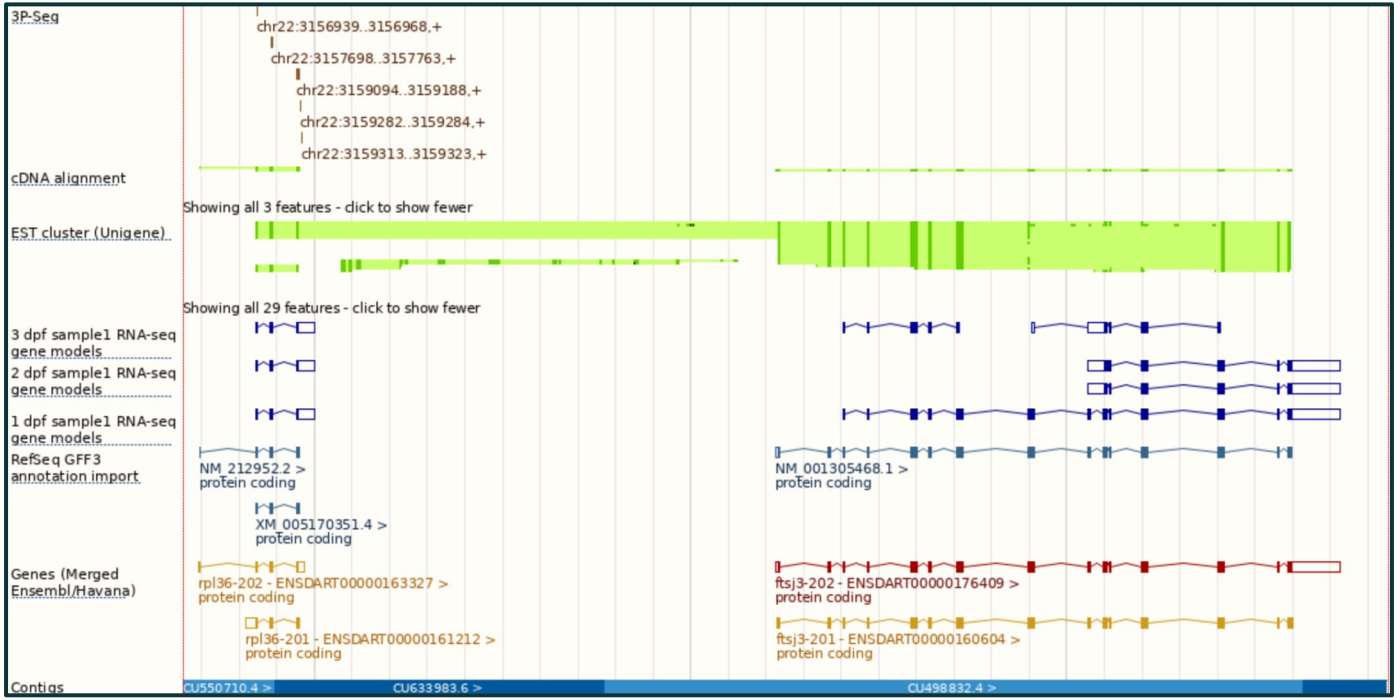
Custom Tracks

- Go to "22:3153000-3217000" (reverse strand)



Custom Tracks

- Go to "22:3153000-3217000" (forward strand)

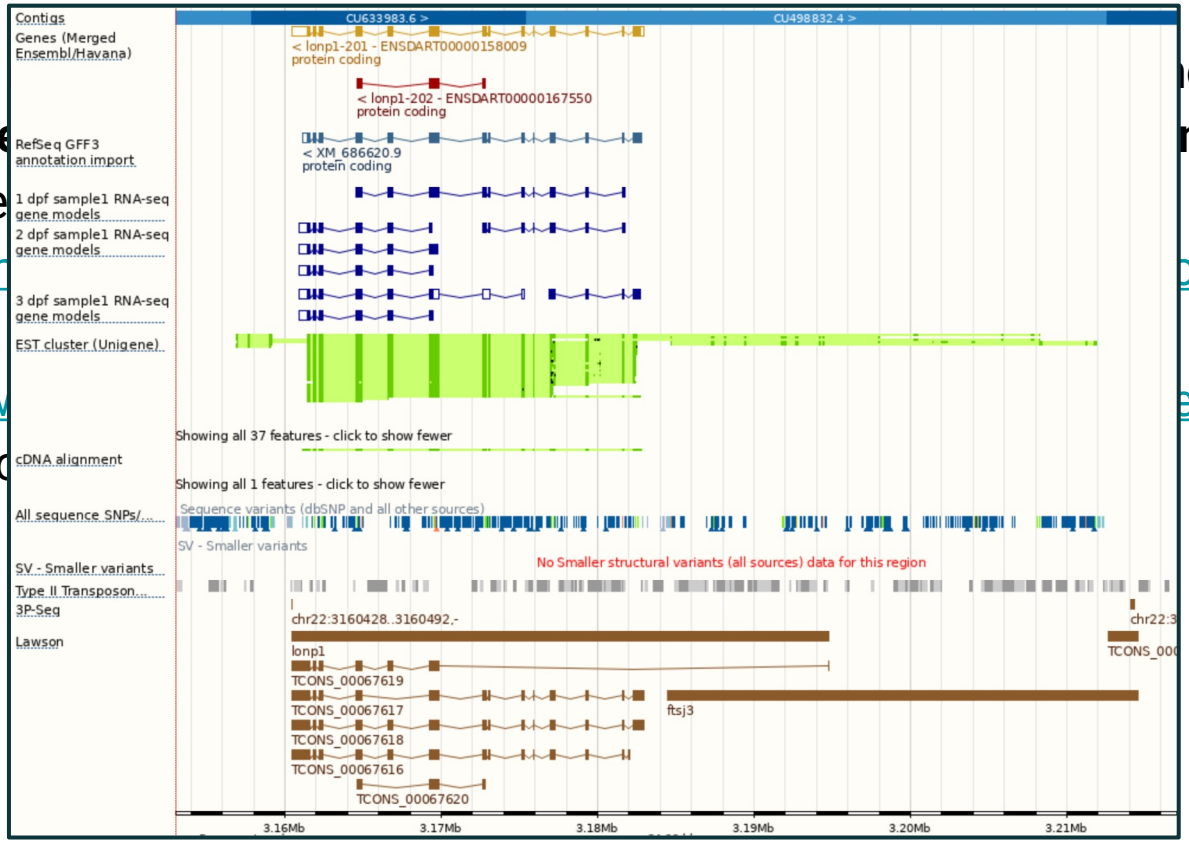


Custom Tracks - Lawson Lab Annotation

- Lawson *et al.* (2020) “**An improved zebrafish transcriptome annotation for sensitive and comprehensive detection of cell type-specific genes**” eLife 9:e55792
- www.umassmed.edu/lawson-lab/reagents/zebrafish-transcriptome/
- Add: https://www.umassmed.edu/globalassets/lawson-lab/downloadfiles/v4.3.2.gtf
- Large, so Ensembl will be slow - disable or delete when done

Custom Tracks - Lawson Lab Annotation

- Lawson **…notation for sensitive…nes”** eLife 9:e…
- www.um…ome/
- Add: https://w…es/v4.3.2.gtf
- Large, sc…



Exercise 3

- Do Exercise 3 - “exploring data”
- Covers:
  - BioMart
  - Making BED files
  - Finding candidate genes
  - Finding orthologues
- Go to mbl2023.buschlab.org

Thank You!

Any questions?