

Module 2 – Maps and Genome Sequence

i. The Ensembl Genome Browser

Caveat: At the time of writing this tutorial, Zv6 had not been released with a full gene build yet. All following examples are therefore taken from the Zv5 Ensembl. If you're trying to work through the examples yourself, please be aware of the difference in the scaffold naming ('Zv5_...' versus 'Zv6_...').

Aims

- Explain the source for the data in Ensembl
- Introduce the Ensembl browser
- Show the different Ensembl views with examples

Introduction

Ensembl is a joint project of the European Bioinformatics Institute (EBI) and the Wellcome Trust Sanger Institute, funded mainly by the Wellcome Trust, with additional funding from EMBL and NIH-NIAID. Ensembl provides easy access to genomic information with a number of visualisation tools.

The Ensembl site provides automatic baseline annotation of the latest assembly sequence, including gene, transcript and protein predictions. The annotation is integrated with external data sources, such as ZFIN for the zebrafish site. The latest zebrafish assembly is Zv6, which was released on March 31st, 2006.

The key Ensembl web pages are called Views (e.g. GeneView, TextView, MapView, and ContigView). The Ensembl web site gives you the opportunity to directly download data, whether it is a DNA sequence of a genomic contig you are trying to identify novel genes in, or positions of SNPs in a gene you are working on. There is also an FTP site which you can use to download large amounts of data from the Ensembl database, as well as a data mining tool (BioMart, see section 6) which allows flexible and rapid retrieval of information from the databases. There are many ways you can access the data in Ensembl depending on your needs and these are explained here and in other sections.

The Ensembl site is at:

<http://www.ensembl.org>

On this page you will find links to all Ensembl species, documentation, search facilities, downloads and other related links. All Ensembl pages have a tool bar on the left-hand side with quick-access links to several resources and facilities.

The screenshot shows the Ensembl website interface. A yellow box labeled "quick-access menu" points to the left-hand navigation menu. Another yellow box labeled "zebrafish" points to the "zebrafish" link in the "Other chordates" section of the "browse a genome" area. The "quick-access menu" includes sections for "Use Ensembl to...", "Docs and downloads", "Other links", and "Home". The "browse a genome" section is divided into "Mammals", "Other chordates", and "Other eukaryotes". The "zebrafish" link is highlighted in the "Other chordates" section.

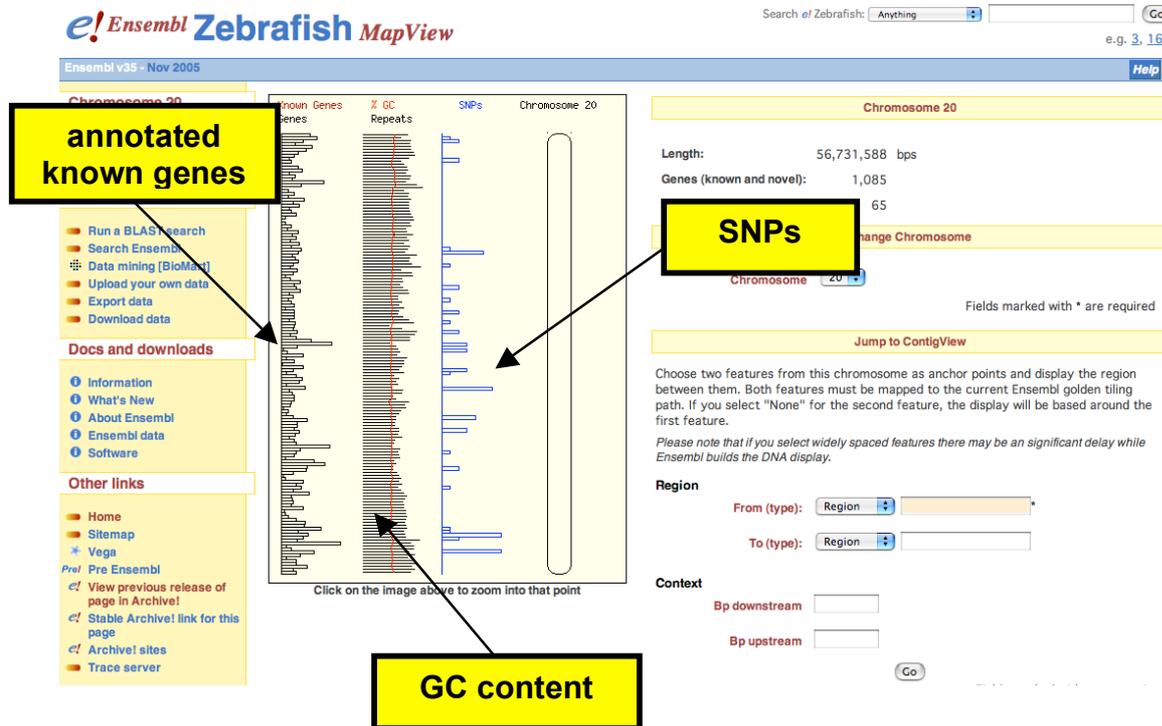
From the main Ensembl site you can access the zebrafish site by clicking on the appropriate species button. As soon a new assembly is released the sequence is made available as a pre-Ensembl site. This includes valuable information such as EST and UniProt alignments and *ab initio* predictions. The main missing data are the Ensembl genes and Ensembl ESTgenes. A full Ensembl dataset for a new assembly is typically made public a couple of months after the assembly release date.

The screenshot shows the Ensembl Zebrafish website. A yellow box labeled "zebrafish chromosomes" points to the "Karyotype" section. The "Karyotype" section displays a karyotype visualization of zebrafish chromosomes, numbered 1 through 25. Below the karyotype, there is a search bar for "Chromosome" and "From (bp)" and "To (bp)" fields, with a "Go" button. The "Explore the Zebrafish genome" section includes "What's New in Ensembl 35" and "Karyotype".

MapView and ContigView

This zebrafish Ensembl page provides various access points to the assembly sequence. For example you can browse a particular chromosome. The

chromosomes are linked to the **MapView** pages. The figure below shows the MapView for chromosome 20.



A MapView page plots the gene and SNP density and GC content. From this page you can zoom in to a more detailed display called ContigView by clicking on the schematic figure representing the chromosome.

ContigView can be considered the central view of the Ensembl web site. It shows the fragments (contigs, clones, etc) that make up a genome assembly. It allows you to scroll along entire chromosomes, whilst viewing the annotated features within a selected region in detail.

A ContigView page is divided into four panels: a chromosome overview, a zoomed-in **overview** of the region in the chromosome you are browsing, a **detailed view** showing features and a **basepair view** that goes down to individual bases. In order to continue with this module, jump to the region under the accession BX004766 (in chromosome 20) with start coordinate 1 and end coordinate 200000. (Use the text box provided to enter these coordinates.)

The image shows a screenshot of the Ensembl Zebrafish ContigView interface. The page title is "Ensembl Zebrafish ContigView" and it includes logos for Sanger and EBI. The main navigation bar contains links for "Zebrafish", "What's New", "Text Search", "Blast Search", "Map Search", "Export Data", "Download", and "Archive sites". A search bar contains the text "[e.g. Zv4_NA18430, Zv4_NA3486]".

The interface is divided into several sections:

- Overview:** Shows a genomic map of Chromosome 20 with contigs and markers. A callout box labeled "Overview" points to this section.
- Detailed view:** Provides a zoomed-in view of a specific region (25,272,803 to 25,357,225). It includes a "Features Menu" with options like "Compare", "DAS Sources", "Repeats", "Decorations", "Export", "Jump to", and "Image size". A callout box labeled "Detailed view" points to this section.
- Ensembl genes:** Displays gene models for Ensembl, including transcripts and proteins. A callout box labeled "Ensembl genes" points to this section.
- EST genes:** Shows EST (Expressed Sequence Tag) data. A callout box labeled "EST genes" points to this section.
- Basepair view:** Shows the raw DNA sequence (A, C, G, T) and amino acid translations. Restriction enzymes are also indicated. A callout box labeled "Basepair view" points to this section.

At the bottom of the page, there is a date "Date: Wed Jul 6 19:57:49 2005", an "Archive" link, and a "Help Desk / Suggestions" link.

The Features menu in the detailed view controls the tracks you can visualise in the panel. Tracks can be turned on and off and the features can be collapsed to simplify the view. Spend some time on this page trying the different menus and studying the displayed features. Observe that there are two tracks for predicted genes: Ensembl transcripts and EST transcripts. (If these features are not visible verify that the corresponding tracks are selected in the menu.)

GeneView, TransView, ExonView and ProtView

Another important view in Ensembl I are the **GeneView** pages with information about the Ensembl predicted genes. In the ContigView page above there is a predicted transcript on the forward strand called **jag2**. Clicking on this transcript displays a pop-up window with several options. Follow the link labelled Ensembl Gene: ENSDARG0000021389. Below we only show the top of the GeneView page for jag2; scroll down to view all the information available.

GeneView provides annotation and supporting evidence for the selected gene. The annotation consists of transcripts, homologies to other species, known and predicted proteins and domains, and links to external documentation. In this example, jag2 is a gene known to ZFIN and so a link to the corresponding external page is provided. The annotation for jag2 is based on 2 transcripts. In the Transcripts sections there are links to the corresponding TransView pages. Click on the link labelled "Transcript info" for the first one with identifier ENSDART0000024922.

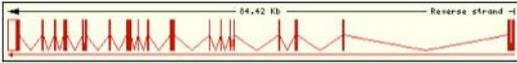
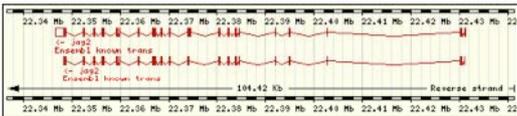
Ensembl Gene Report for ENSDARG0000021389

Gene	jag2 (ZFIN) click here		
Ensembl Gene ID	ENSDARG0000021389		
Genomic Location	This gene can be found on Chromosome 20 at location 22,346,74 . This start of this gene is located in Chunk BX004766.9.2000-212782 .		
	jagged 2 isoform 1 Source:RefSeq_peptide:NP_571937		
	Genes were annotated by the Ensembl automatic analysis pipeline using GeneWise models from a protein alignment (with priority given to zebrafish proteins). GeneWise models are further combined with available aligned cDNAs and EST clusters to annotate UTRs.		
Transcripts	ENSDART0000024922	ENSDARP0000010799	jag2 [Transcript info] [Exon info] [Peptide info]
	ENSDART0000049586	ENSDARP0000049585	jag2 [Transcript info] [Exon info] [Peptide info]
	<p>Features</p> <p>Chr. 20</p> <p>Length 104.42 Kb</p> <p>DNA(contigs)</p> <p>Ensembl trans.</p> <p>Length 104.42 Kb</p>		
Orthologue Prediction	The following gene(s) have been identified as putative orthologues by reciprocal BLAST analysis:		
Species	Type	dN/dS	Gene identifier
<i>Homo sapiens</i>	UBRH		ENSG00000184916 (JAG2) [MultiContigView] [Align]
			Jagged-2 precursor (Jagged2) (HJ2). [Source:UniProt/SWISSPROT;Acc:Q9Y219]
<i>Mus musculus</i>	UBRH		ENSMUSG00000002799 (Jag2) [MultiContigView] [Align]
			jagged 2 [Source:MarkerSymbol;Acc:MG1098270]

Annotations in the image:

- Transcript info**: Points to the transcript identifier ENSDART0000024922.
- Exon info**: Points to the [Exon info] link for the first transcript.
- Peptide info**: Points to the [Peptide info] link for the first transcript.
- Transcript Structure**: Points to the Features section of the transcript details.

Ensembl Transcript Report

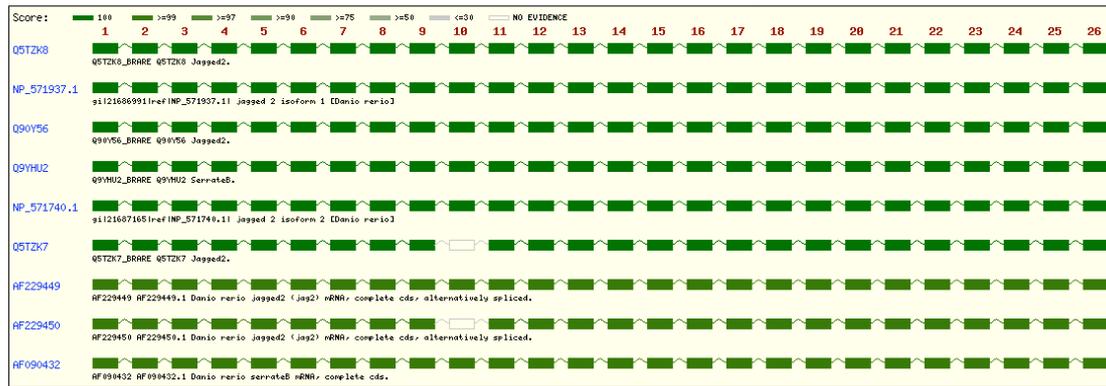
Transcript	jag2 (ZFID ID) (to view all Ensembl genes linked to the name click here)
Ensembl Transcript ID	ENSDART0000024922
Transcript Information	Exons: 26 Transcript length: 5,436 bps Translation length: 1,254 residues This transcript is a product of gene: ENSDARG0000021389
Genomic Location	This transcript can be found on Chromosome 20 at location 22,346,747-22,431,169 . This start of this transcript is located in Chunk BX004766.9.2000-212782 .
Description	jagged 2 isoform 1 Source: RefSeq_peptide NP_571937
Prediction Method	Genes were annotated by the Ensembl automatic analysis pipeline using GeneWise models from a protein alignment (with priority given to zebrafish proteins). GeneWise models are further combined with available aligned cDNAs and EST clusters to annotate UTRs.
Similarity Matches	This Ensembl entry corresponds to the following database identifiers: RefSeq peptide: NP_571740.1 [Target %id: 99; Query %id: 99] [align] NP_571937.1 [Target %id: 99; Query %id: 99] [align] RefSeq DNA: NM_131665.1 [Target %id: 93; Query %id: 98] [align] NM_131862.1 [Target %id: 99; Query %id: 99] [align] Predicted UniProt/TrEMBL: Q5T2K8_BRARE [Target %id: 100; Query %id: 100] [align] Q90Y56_BRARE [Target %id: 99; Query %id: 99] [align] Q9YHU2_BRARE [Target %id: 99; Query %id: 99] [align] EntrezGene: 140422 EMBL: AF090432 [align] AF229449 [align] BX004766 [align] IPI: IPI00500671.1 [Target %id: 99; Query %id: 99] IPI00501275.2 [Target %id: 100; Query %id: 100] Protein ID: AAC98354.1 [align] AAL08214.1 [align] CAH69087.1 [align] UniGene: Dr_8287 [Target %id: 99; Query %id: 99] ZFIN ID: jag2 Affymx Microarray Zebrafish: Dr_8287.1.S1_a_at
GO	The following GO terms have been mapped to this entry via UniProt: GO:0001889 [liver development] GO:0005509 [calcium ion binding] GO:0007154 [cell communication] GO:0016020 [membrane]
InterPro	IPRO01438 Type II EGF-like signature - [View other genes with this domain] IPRO01881 EGF-like calcium-binding - [View other genes with this domain] IPRO01687 ATP/GTP-binding site motif A (P-loop) - [View other genes with this domain] IPRO01774 Delta/Serrate/lag-2 (DSL) protein - [View other genes with this domain] IPRO00742 EGF-like, subtype 2 - [View other genes with this domain] IPRO01093 IMP dehydrogenase/GMP reductase - [View other genes with this domain] IPRO00152 Aspartic acid and asparagine hydroxylation site - [View other genes with this domain] IPRO06209 EGF-like - [View other genes with this domain] IPRO11651 Notch ligand, N-terminal - [View other genes with this domain]
Protein Family	ENSE0000000046 : PRECURSOR This cluster contains 29 Ensembl gene member(s)
Transcript structure	
Transcript neighbourhood	
Transcript sequence	GTGATCAGACCGAGGAGAGATCAGCACAGACATCACCCGCAACACACCACCGTCGT GAAATTTTGCATGTCAGGAACGGAGGATCTGTGCCGGTCATCGGCCGTTTTCATCTT GCCTTATAACACATCAAAATCGCCGATGTGGAAATGTATCAGGATTAAGAAATGGCTC CAAATCGCTGCTGCTGTAAACAAATGTGGACGAAGGTGTCCCAAGCTCTGTGCTA GAGTTCACCTGATTTCTGTAGAAAGTAAAGCGTGGTGGGAGCGGGAAATG GACAGCACCGGAACCTCAAGCAGCCGCTGCGTGGCGGACGAGTGGCACTACTT AAAGTGTCTGAAGGAGTACCAGTCTGAAGTCAACCACTGGACAGTGCACCTTCGCG TCTGGATCACCAGCTCTTGGTGAATAATAATTTCTTTAAGACCGCAAAAACAGC CCCAAGCAAAAGAGCGGTGGGAAGGATCATATCCCTTTGCACTGCTGCGCCGGA TCTCACACTCATCTTGAAGCTTGGGACTGGGATAACTCCACTCAGAAACATGTTGAA GAAATTTGATCGAACGGACATTCACCAAGCATGGTAAACCCCGGCAACCACTGGCAG TCCATCCGACCTTGCATCAGCGCCACATTTAGATCCGATCCGCTGTCAGGTGTGAT GAGATTAATGAGGTAAGTCAACCAACTGTGCGCCAGGATGACTACTCTGGT CATACCGATGCACTCACTGGAATATTTGTGTCTTGTGATGCTGGATGGGAGGAC TTCGGACAGCGATCTCAAGCAGGGCTGTAATCTGATTCACGGAGGCTGTGGGTCCT GGAGATGCAAGTGCACACTACCGCTGGCAGGGCAGTCTGGCAGAGTGTCTACCTTAT CTTGGCTGTTGGCAGGTAACCTGTGTATGCTTGGCAATGCACTGTGAGAAAGACTGG CGCCGCTCTCTGGGATAAAGATCTGAGACTGCGCCAGCAGTACTCTGCTGTGAAAT GGTGAACCTGCATGAACTCTGAACCGGATGAATAAATCTGCTCTCCGAAAGGCTAC

cDNA

TransView provides annotation and supporting evidence for the selected transcript (structure, transcribed proteins, Gene Ontology and InterPro associated entries). The Transcript report panel provides a top-level summary of the transcript, with links to its genomic location, alignments to sequences in external databases, and export options. Underneath the report, the cDNA sequence of the transcript can be shown with codons, peptide sequence and/or SNPs highlighted.

Support Evidence

The supporting evidence below consists of the sequence matches on which the exon predictions were based and are sorted by alignment score.



Finally from the links labelled “Peptide info” in the GeneView page we can visit the ProtView page for the associated translation.

Ensembl v35 - Nov 2005

Ensembl Protein Report

Peptide jag2 (ZFIN ID ID) (to view all Ensembl genes linked to the name [click here](#))

Ensembl Peptide ID ENSDARP00000010799

Translation information This protein is a translation of transcript [ENSART00000024922](#), which is a product of gene [ENSARG00000021389](#).

Genomic Location This peptide can be found on Chromosome 20 at location [22,348,271-22,431,022](#). This start of this peptide is located in [Chunk BX004766.9.2000-212782](#).

Description jagged 2 isoform 1 [Source: RefSeq_peptide NP_571937](#)

Prediction Method Genes were annotated by the Ensembl automatic analysis pipeline using GeneWise models from a protein alignment (with priority given to zebrafish proteins). GeneWise models are further combined with available aligned cDNAs and EST clusters to annotate UTRs.

InterPro [IPR001438](#) Type II EGF-like signature - [\[View other genes with this domain\]](#)
[IPR001881](#) EGF-like calcium-binding - [\[View other genes with this domain\]](#)
[IPR001687](#) ATP/GTP-binding site motif A (P-loop) - [\[View other genes with this domain\]](#)
[IPR001774](#) Delta/Serrate/jag-2 (DSL) protein - [\[View other genes with this domain\]](#)
[IPR000742](#) EGF-like, subtype 2 - [\[View other genes with this domain\]](#)
[IPR001093](#) IMP dehydrogenase/GMP reductase - [\[View other genes with this domain\]](#)
[IPR000152](#) Aspartic acid and asparagine hydroxylation site - [\[View other genes with this domain\]](#)
[IPR006209](#) EGF-like - [\[View other genes with this domain\]](#)
[IPR011651](#) Notch ligand, N-terminal - [\[View other genes with this domain\]](#)

Protein Family [ENSE0000000046](#) : PRECURSOR
This cluster contains 29 Ensembl gene member(s)

Protein Features

Protein Sequence

```
MWNCIRIRNWLPIACLLLTMTKVSQSSGYFELQLIAVENVNGELMDGECDCSTRNSQDQ
RCVRECDCTYFVKLKEYQSEVTTTGGCTFESGSTDVIGNIISFKTAKNSPKRTDVGK
IIPFPFAMPRFYLILLAWQKSTQNSGENIIRIIRIIRIIRIIRIIRIIRIIRIIRIIRI
HISYRIIRVRCDENYGSCKKQCRPRDDYFHYRCDPFGNIVCLDGNNGEDCRDAICKQG
CNLIHGCCAVPGECKCNYGWQGFCDCELPYGCILRGTCVHPWQCTCERNMGLLQDKDL
NYCOTSHPCVNGTCCMSSEPDFYKACFEYSGKNCEIARACVENPCANGGTCHEVPTG
FECRCFPQHEGFTCAKMDKDCASSPCAQGGCTCIDLNGFECVCFQWVGTQCIIDANICM
GRPCVNASCKRMIGYKCCDFGGAGGKCCILNCGCGGCGMGATCKELVNGVYSCCP
```

ProtView shows information about the structure and function of the encoded protein in the transcript's report with external links to various databases like Pfam, Prosite, etc...

ExportView

ExportView lets you download/dump data. All the features for a genomic region may be downloaded or exported to several formats (for example, FASTA, GenBank or EMBL-style flat file, as a feature list or an image). The ExportView pages are accessible from the link 'Export data' in the left-hand side menu from any of the pages above.

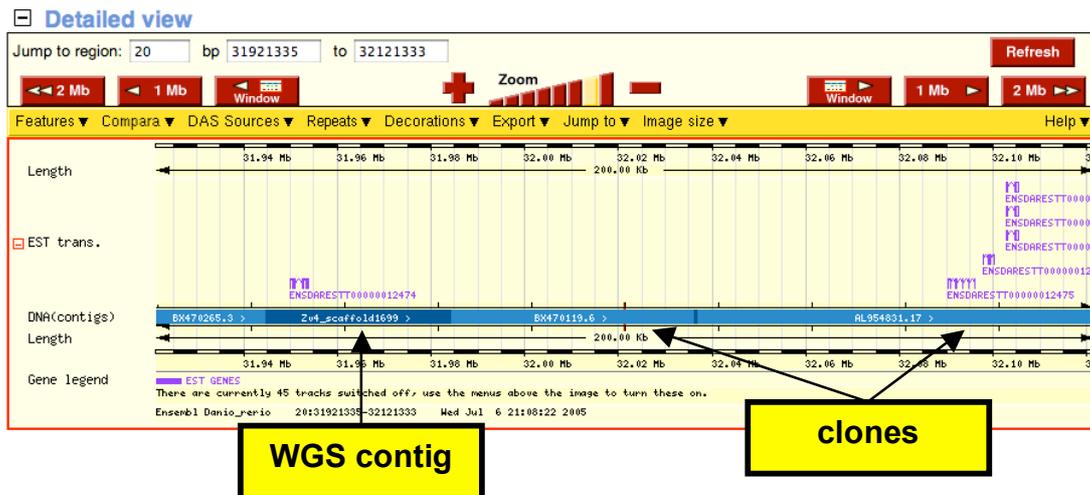
Zebrafish assembly in Ensembl

The sequence in the *Danio rerio* Ensembl database is the latest assembly release with automatic annotation. The genomic sequence released is based on all the sequenced clones with remaining gaps covered by contigs from a whole genome shotgun (WGS) assembly. The WGS fragments are placed in those gaps using a mixed strategy that looks at sequence similarity and other anchors as BAC-ends and markers. This placement is hard to perform without errors - mainly due to the presence of mis-joins in the WGS assembly and duplicates. It is even more difficult to place sequence where there is no sequenced clone or marker to use as an anchor.

In this context the user has to evaluate the data with a critical eye. In particular when the sequence of interest is known to the community but it is wrong in the assembly. There are three kinds of scaffolds and these are, in order of quality from best to worst:

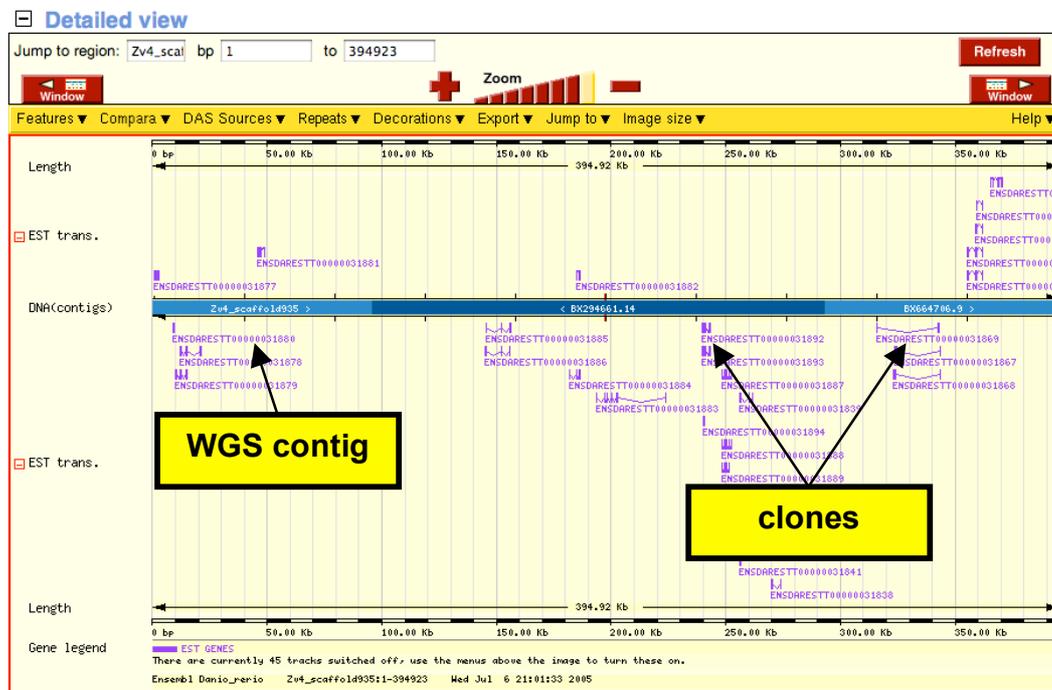
1. scaffolds that have been attached to chromosomes (they may contain sequenced clones),
2. scaffolds that can be aligned to clones but the physical map cannot assign a chromosome yet (they may contain sequenced clones), and
3. NA (non-attached) scaffolds that corresponds to WGS contigs that could not be placed in the map (they don not contain sequenced clones).

Zv5_scaffold1699 is an example of category 1 above. This scaffold is placed in chromosome 20.



In the detailed view for this page there is a genomic region labelled BX470265.3. This is the accession number of a sequenced clone. The region labelled Zv5_scaffold1699 is a WGS supercontig. This is of lower quality than the sequenced clone (and may contain gaps represented by a sequence of Ns).

Zv5_scaffold935 is an example of a region that is part of the map but, when the assembly was built, did not have a placement in a chromosome (category 2). This example shows that the region contains some sequenced clones as shown by the presence of their accession numbers.



Finally a scaffold from category 3 is Zv5_NA10. This region does not contain any finished clones.

Exercises

This section introduces the Ensembl browser and some of its basic views. In other section we will study more advanced features like the compara database and Blast/SSAHA search facilities. The user is encouraged to navigate the site and experiment with the different views discussed above.

1. Find the GeneView page for *jag2* (Ensembl gene), and scroll down to the first 'Transcript/Translation Summary'. As *jag2* has been identified in Zv6 you can use this gene name in a text search box.
2. Examine the genomic context. From GeneView, follow the link 'View gene in genomic location' to ContigView.
3. Customise the display of ContigView selecting different tracks and comparing the data from different tracks.
4. In ContigView zoom in to examine the data in more detail.
5. Export a file containing the cDNA of one of the predicted transcripts for *jag2*.
6. One of the Ensembl tracks displays probes for which ZFIN has an expression pattern page. Search for the mapping of the EST with accession CK685476 and open the corresponding ContigView page. Make sure that the 'expression pattern' track is selected in the 'features' menu. The ContigView page displays a link to the expression pattern page in ZFIN, try it.