Access to genes and genomes with Ensembl

Course Manual

e60

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I) Introduction

Ensembl is one of the world's primary resources for genomic research, a resource through which scientists can access the human genome as well as the genomes of other model organisms. Because of the complexity of the genome and the many different ways in which scientists want to use it, Ensembl has to provide many levels of access with a high degree of flexibility. Through the Ensembl website a wet-lab researcher with a simple web browser can for example perform BLAST searches against chromosomal DNA, download a genomic sequence or search for all members of a given protein family. But Ensembl is also an all-round software and database system that can be installed locally to serve the needs of a genomic centre or a bioinformatics division in a pharmaceutical company enabling complex data mining of the genome or large-scale sequence annotation.

The need for automatic annotation

Recent years have seen the release of huge amounts of sequence data from genome sequencing centres (figure 1). However, this raw sequence data is most valuable to the laboratory biologist when provided along with quality annotation of the genomic sequence.

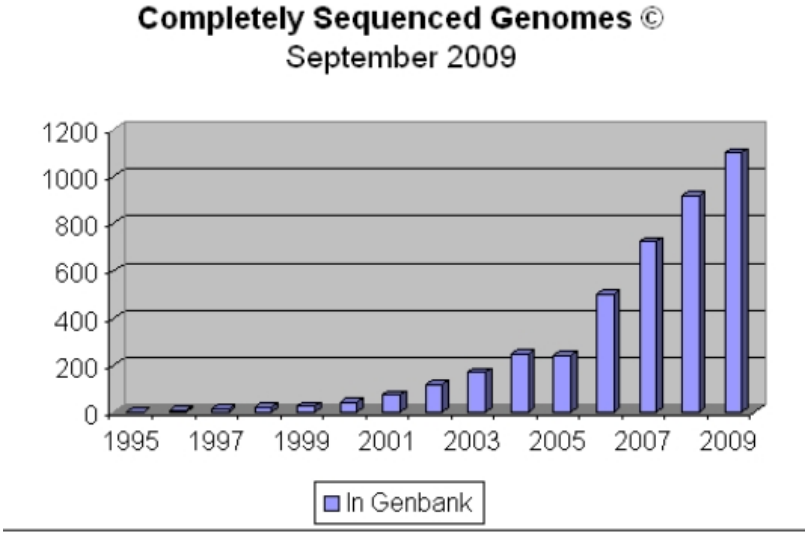


Figure 1. Completely sequenced genomes as of January, 2009 (figure taken from http://www.genomesonline.org).

This information can be the starting point for planning experiments, interpreting Single Nucleotide Polymorphisms, inferring the function of gene products, predicting regulatory sites for gene expression and so on. The

currently agreed 'gold standard' for the annotation of eukaryotic genomes is annotation made by a human being. This so-called “manual annotation” is based on information derived from sequence homology searches, the results of various *ab initio* gene prediction methods and literature searches. Annotation of large genomes (such as mouse and human) that meet this standard is slow and labour intensive, taking large teams of annotators years to complete. As a result, the annotation can almost never be entirely up-to-date and free of inconsistencies (as the annotation process usually begins before the sequencing process is complete). Hence, an automated annotation system is desirable since it is a relatively rapid process that allows frequent updates to accommodate new data. To meet this need, we produced the Ensembl annotation system by observing how annotators build gene structures and condensing this process into a set of rules.

The start of Ensembl

Ensembl's genesis was in response to the acceleration of the public effort to sequence the human genome in 1999. At that point it was clear that if annotation of the draft sequence was to be available in a timely fashion it would have to be automatically generated and that new software systems would be needed to handle genome data sets that were much larger, much more fragmented and much more rapidly changing than anything previous dealt with.

Ensembl was conceived in three parts: as a scalable way of storing and retrieving genomic data; as a web site for genome display; and as an automatic annotation method based around a set of heuristics. It was initially written for the draft human genome, which was sequenced clone-by-clone but has also been successfully used for whole genome shotgun assemblies. The storage and display parts of Ensembl are used for all the genomes currently present in Ensembl, while the automatic gene annotation has been run for most of the genomes with the exception of Takifugu, Tetraodon, Fruitfly, *C. elegans* and Yeast.

Over the past few years Ensembl has grown into a large scale enterprise, with substantial computing resources enabling it to process and provide live database access to currently more than 25 different genomes (figure 2) and a bimonthly update frequency to its website. It has a large community of users in both industry and academia, using it as a base for their individual organisation’s experimental and computational genome based investigations, some of which maintain their own local installations.

Ensembl is a collaboration between the European Bioinformatics Institute (EBI) and the Wellcome Trust Sanger Institute, both located on the Wellcome Trust Genome Campus in Hinxton, Cambridge, UK. Ensembl is funded principally by the Wellcome Trust, with additional funding from the European Molecular Biology Laboratory (EMBL), the National Institutes of Health – National Institute of Allergy and Infectious Disease (NIH-NIAID) and the Biotechnology and Biological Sciences Research Council (BBSRC).

The Ensembl software and database system

As a software/database system Ensembl can be best described as a hybrid of a scripting programming language (Perl) and a relational database (MySQL, pronounced “My Ess Que Ell”).
Ensembl Perl software inherits from a tradition of biological object-design developed through BioPerl (http://www.bioperl.org/). This means that developers at Ensembl aimed at creating reusable pieces of software that would faithfully describe biological entities such as gene, transcript, protein, genomic clone or chromosome. Rules of usage and design of Ensembl and BioPerl objects can be best learned while using them, browsing their code and through a bit of trial-and-error. There is a comprehensive BioPerl tutorial available at the BioPerl website.
The Ensembl database is based on a relational database called MySQL. SQL in MySQL stands for ‘Structured Query Language’, a universal database programming language shared by many relational databases. Because MySQL is available free of charge for non-commercial developers, every academic centre can install its own local copy of MySQL (MySQL server) and download Ensembl data from the Ensembl ftp site. Simple queries of the database can be handled using the SQL language (see appendix), but for complex queries demanded by most biological analyses the Ensembl MySQL server is best accessed using Ensembl Perl objects.

The Ensembl annotation pipeline

The Ensembl analysis and annotation pipeline is based on a rule set of heuristics that a human annotator would use. All Ensembl gene predictions are based on experimental evidence, which is imported via manually curated UniProt/Swiss-Prot, partially manually curated NCBI RefSeq and automatically annotated UniProt/TrEMBL records. Untranslated regions (UTRs) are annotated to the extent supported by EMBL mRNA records. As there is no guarantee that UTR sequences in EMBL records are complete there is similarly no guarantee that the Ensembl genome analysis and annotation pipeline has enough biological evidence to predict complete UTR regions. For a limited number of species regulatory regions are annotated, but this annotation isn’t very extensive yet as the set of well-characterised promoters is still small and there is currently no algorithm yielding reliable results on a genomic scale.

The Ensembl website

Ensembl provides access to genomic information with a number of visualisation tools. The Ensembl website gives you the possibility to directly download data, whether it is the 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. The key Ensembl web pages are covered in the web-site walk-through. An updated version of the website is released bimonthly. Old versions are accessible on the ‘Archive!’ website, dating back two years. Apart

from that the 'Pre!' website provides displays of genomes that are still in the process of being annotated. There is also an ftp site to download large amounts of data from the Ensembl database, as well as the data-mining tool BioMart, that allows rapid retrieval of information from the databases, and BLAST/BLAT sequence searching and alignment.

Further reading

Flicek, P. *et al.*
Ensembl 2011
Nucleic Acids Res. Advanced Access *(Database Issue)*
http://nar.oxfordjournals.org/content/early/2010/11/02/nar.gkq1064.full

Xosé M. Fernández-Suárez and Michael K. Schuster
Using the Ensembl Genome Server to Browse Genomic Sequence Data.
UNIT 1.15 in *Current Protocols in Bioinformatics*, Jun 2010.

Giulietta M Spudich and Xosé M Fernández-Suárez
Touring Ensembl: A practical guide to genome browsing
BMC Genomics 2010, 11:295 (11 May 2010)

Vilella, A.J. *et. al*
EnsemblCompara GeneTrees: Analysis of complete, duplication aware phylogenetic trees in vertebrates.
Genome Res. 2009 Feb 19(2):327-35

Smedley, D. *et al*
BioMart – biological queries made easy
BMC Genomics 2009 Jan 14;10:22

Flicek, P. *et. al*
Ensembl 2008
Nucleic Acids Res. Jan 2008; 36: D707 - D714

Spudich, G., Fernández-Suárez, X. M., and Birney, E.
Genome Browsing with Ensembl: a practical overview
Brief Funct Genomic Proteomic, 2007 Sept; 6: 202-219

Birney, E. *et al*.[1]
An Overview of Ensembl.
Genome Research 14(5): 925-928 (2004)

Ashurst, J. L. et *al.*
The Vertebrate Genome Annotation (Vega) database.
Nucl. Acids Res. 33:D459-D465 (2005)

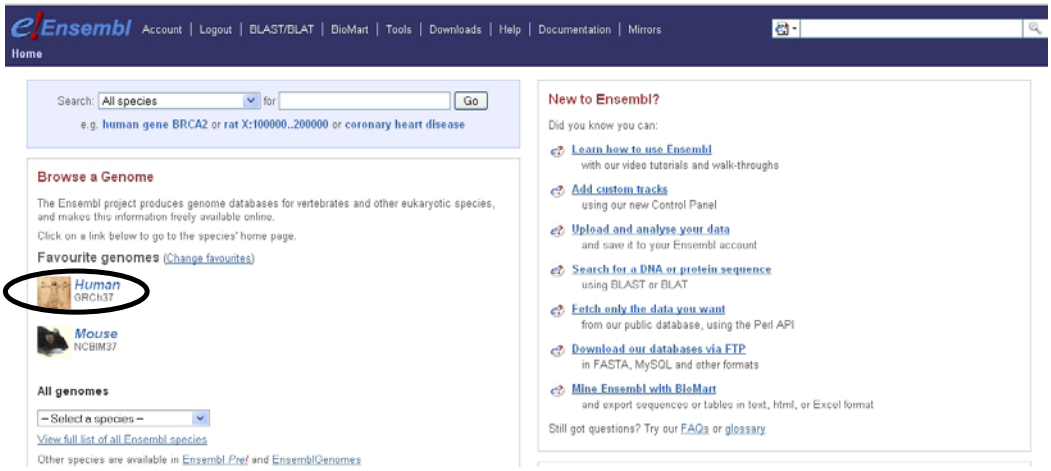
[1] This paper was part of the May 2004 issue of *Genome Research* which included an Ensembl special covering detailed aspects of the Ensembl web site, the underlying scalable database system for storing genome sequence and annotation information, as well as the automated genome analysis and annotation pipeline

II) WALKING THROUGH THE WEBSITE

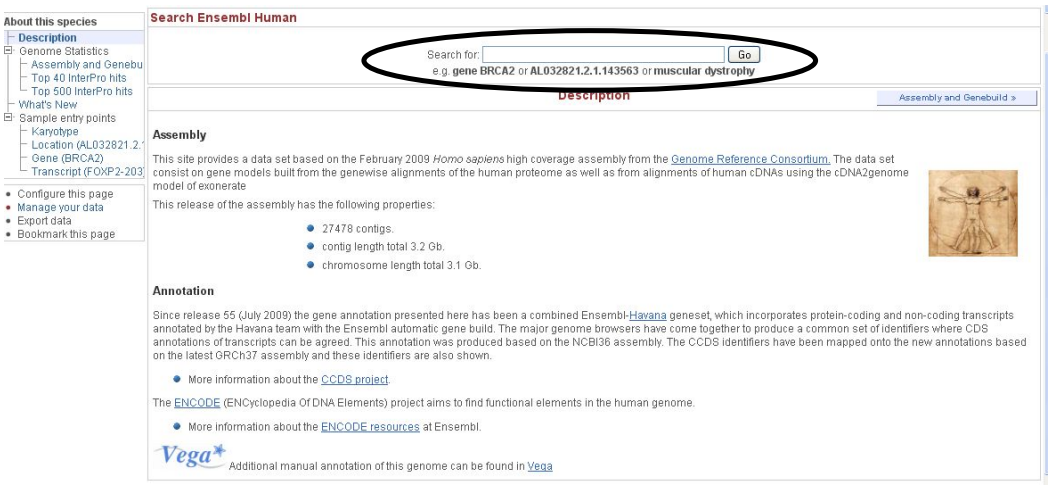
The instructor will guide you through the website using the human *rhodopsin* **(RHO)** gene. The following points will be addressed:

- **The Gene Summary tab and gene-related links:**
  - Are there splice variants?
  - Can I view the genomic sequence with variations?
  - Find orthologues and paralogues
- **The Transcript tab and related links:**
  - What is the protein sequence?
  - What matching proteins and mRNAs are found in other databases?
  - Gene Ontology
- **The Location tab and related links:**
  - What's the conservation track?
  - How do I zoom in and change the gene focus.
  - Un-stacking a track (e.g. human cDNAs)
  - Adding a track (i.e. variations)
- **Exporting a sequence and running BLAT/BLAST**

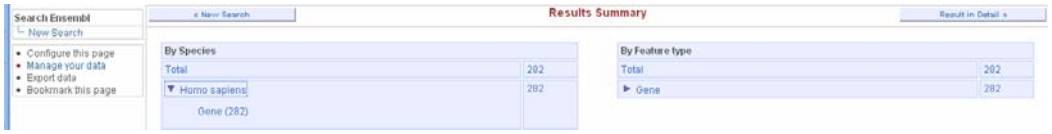
Start by going to ***www.ensembl.org***



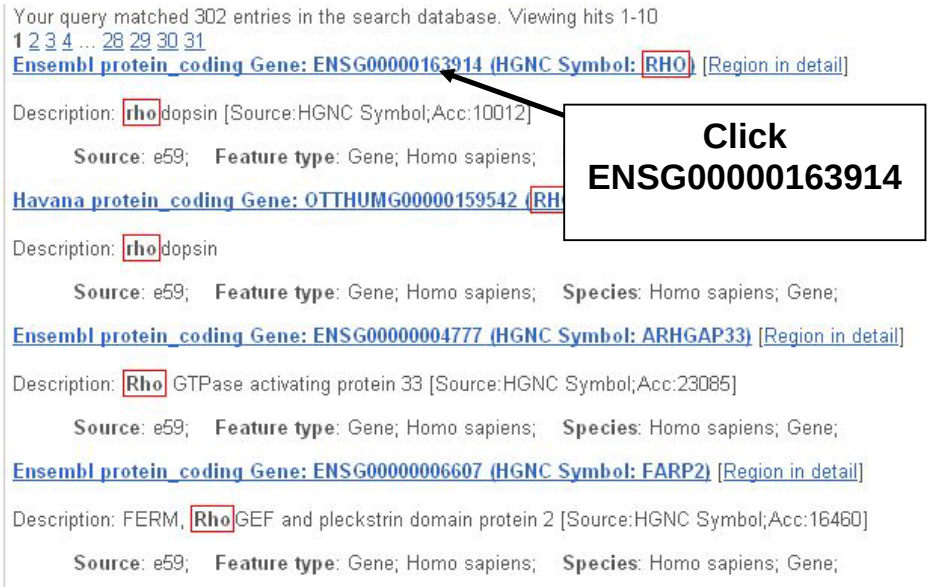
Click on 'Human', or the picture circled above, which brings us to the species home page.



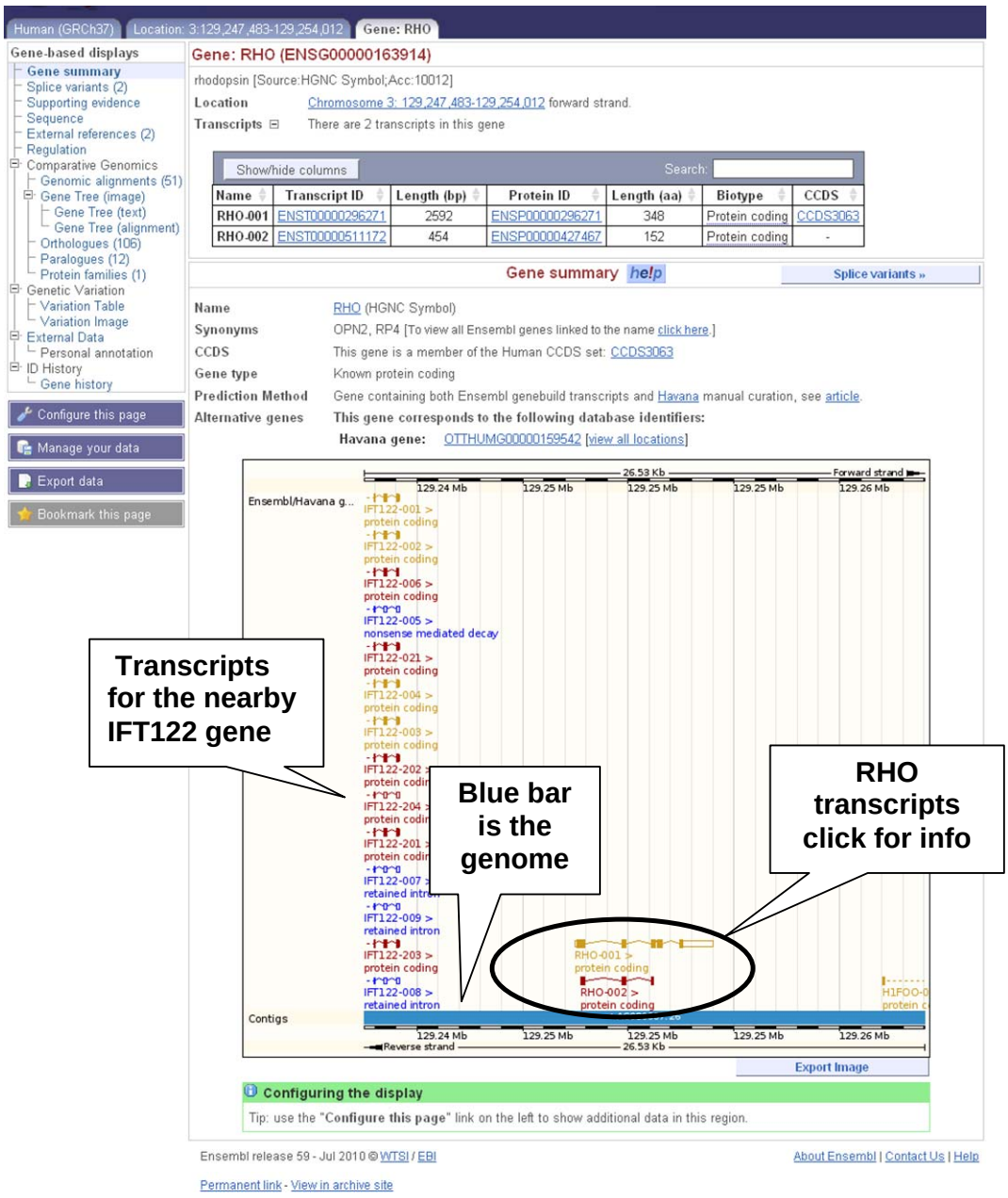
Type 'gene RHO' into the search bar circled above and click the 'Go' button.



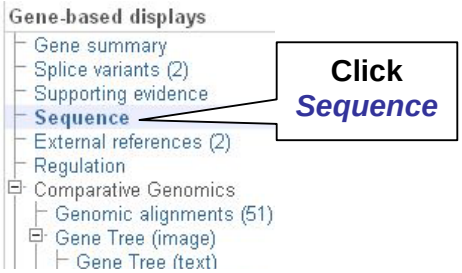
Click the arrow next to Homo sapiens to expand the hits, and click the 'Gene' link when it is revealed.

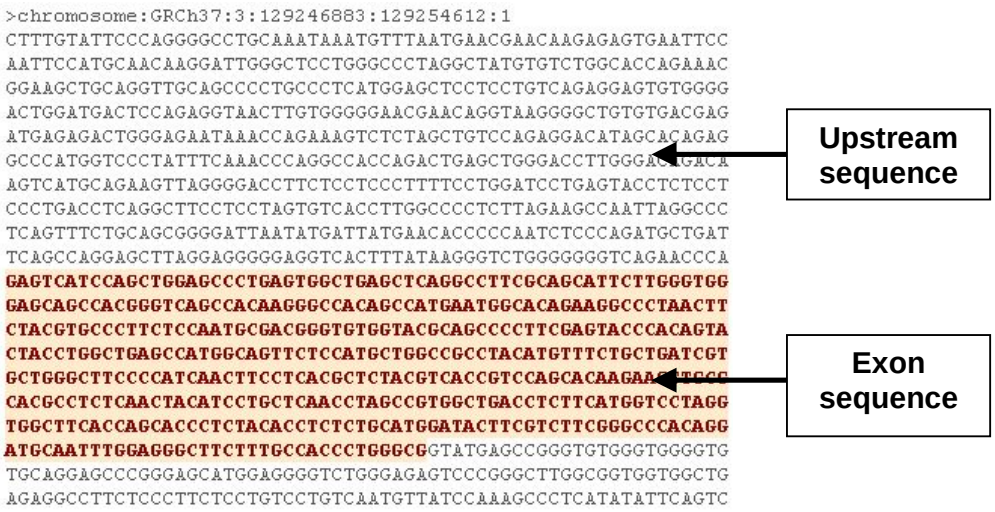


Look through the search results for RHO, the gene symbol. Select the Ensembl gene (i.e. ENSG00000163914), rather than manually curated Havana genes. The following 'Gene' tab should open:

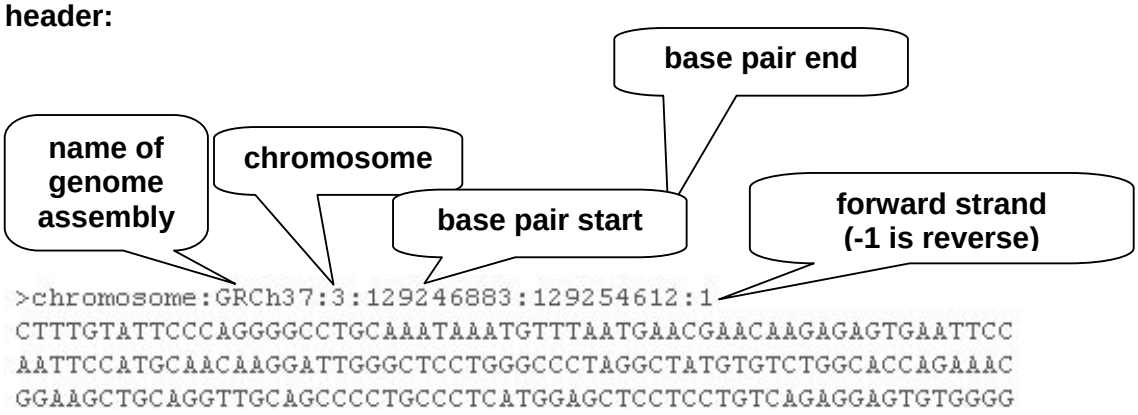


Let's walk through some of the links in the left hand navigation column. How can we view the genomic sequence? Click *Sequence* at the left of the page.



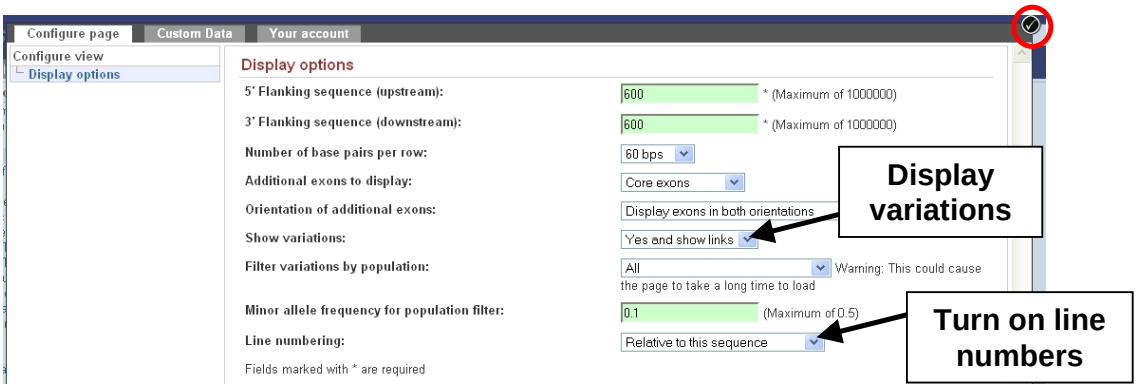


The sequence is shown in FASTA format. Take a look at the FASTA header:



Exons are highlighted within the genomic sequence. Variations can be added with the 

link found at the left. Click on it now.



Once you have selected changes (in this example, display variations and show line numbers) click at the top right (circled in red, above).

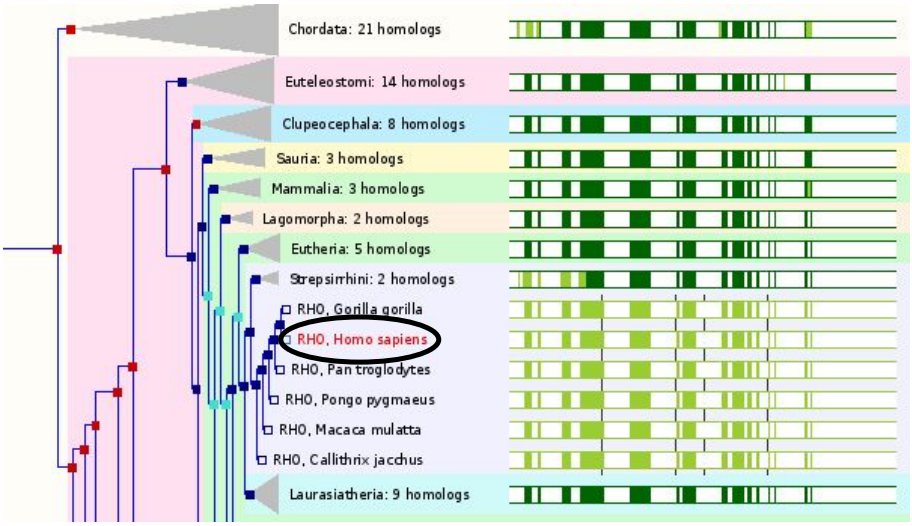
Variations in the sequence are highlighted, and represented by the IUPAC code. The code "R" represents alleles A or G. Links to variation pages (one is circled) are shown at the right. Line numbers have been added.

Now let's click on *Genomic alignments*, to see a nucleotide view of the whole genome alignments. Select the 6 primates, EPO. The EPO pipeline refers to the programs behind the whole genome alignments - click the help button for more.

Click Configure this page at the left. Turn on Conservation regions to "All conserved regions" in the menu. Also, click "6 primates EPO" at the left of the configuration panel. Deselect "Ancestral sequence". Close the menu.

Exons are highlighted in red, conserved nucleotides are highlighted in blue.

Now let's click on *Gene tree (image)*, which will display the current gene in the context of a phylogenetic tree used determine orthologues and paralogues.



Click on any node (square) to reveal the taxonomic level, or to collapse or expand a subtree.

Click the *Orthologues* link at the left of this page to view homologues detected in this tree.

« Gene Tree (image) | Orthologues help | Paralogues »

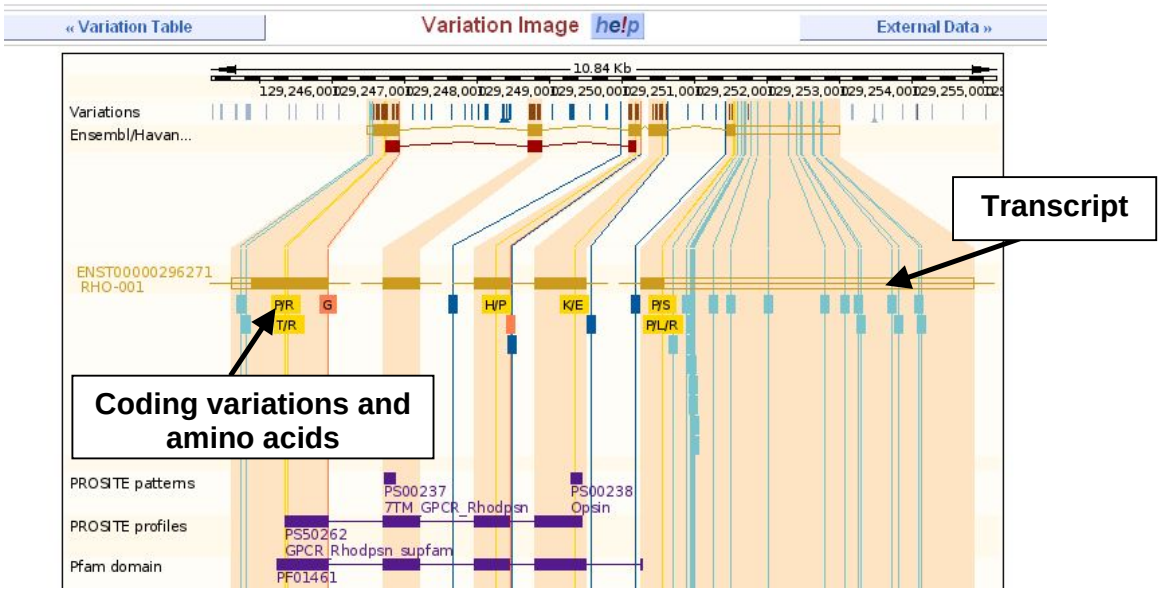
The following gene(s) have been identified as putative orthologues:

Species	Type	dN/dS	Ensembl identifier	Location	Target %id	Query %id	External ref.
Alpaca (*Vicugna pacos*)	1-to-1	n/a	ENSVPAG00000003334 Multi-species view Alignment Gene Tree (image)	GeneScaffold_3012:4867-8438:1	77	77	RHO rhodopsin [Source:HGNC Symbol;Acc:10012]
Anole Lizard (*Anolis carolinensis*)	1-to-1	n/a	ENSACAG00000014258 Multi-species view Alignment Gene Tree (image)	scaffold_163:632250-640401:-1	81	82	OPSD_ANOCA Rhodopsin [Source: UniProtKB/Swiss-Prot; acc: P41591]
Anole Lizard (*Anolis carolinensis*)	Possible ortholog	n/a	ENSACAG00000005789 Multi-species view Alignment Gene Tree (image)	scaffold_24:1173306-1186146:1	39	33	Novel Ensembl prediction No description
Anole Lizard (*Anolis carolinensis*)	Possible ortholog	n/a	ENSACAG00000006891 Multi-species view Alignment Gene Tree (image)	scaffold_44:1171109-1177064:-1	38	28	Novel Ensembl prediction No description
Anole Lizard (*Anolis carolinensis*)	Possible ortholog	n/a	ENSACAG00000008548 Multi-species view Alignment Gene Tree (image)	scaffold_1370:18504-28260:1	44	28	Q9W6K3_ANOCA P opsin [Source: UniProtKB/TrEMBL; acc: Q9W6K3]

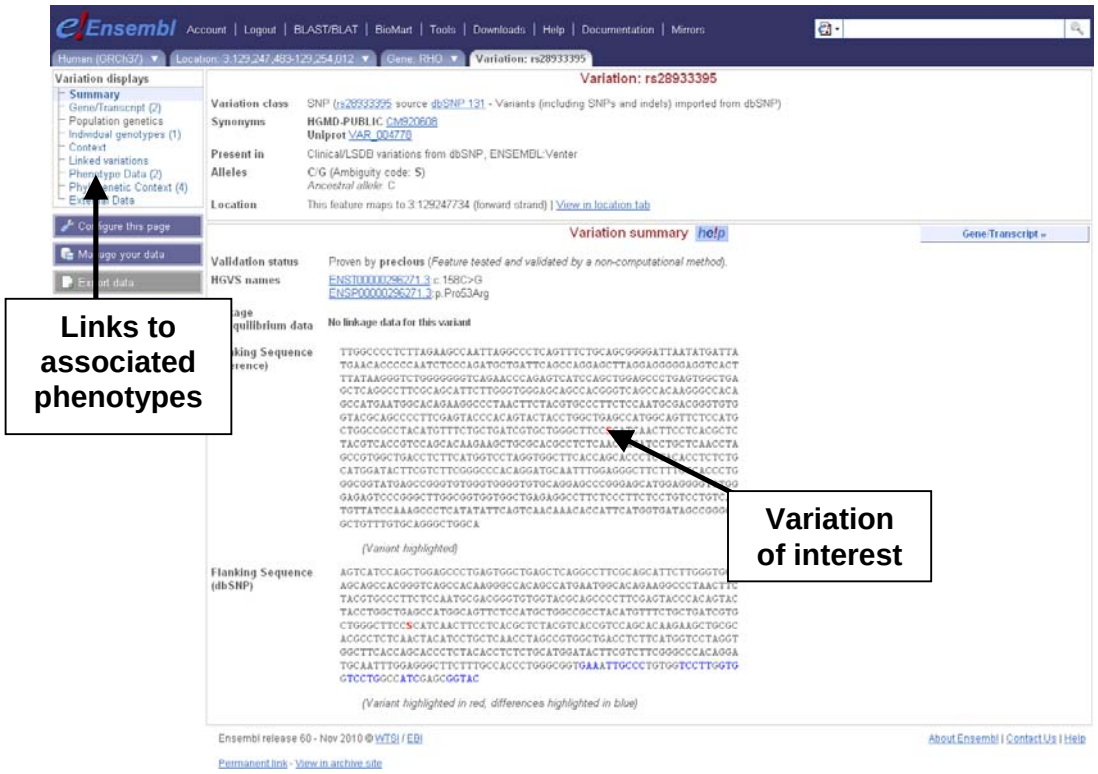
Let's view genetic variation mapped onto all transcripts of a gene.

First click on *Variation table* at the left. Show all the non-synonymous coding variations for this gene, by clicking "show".

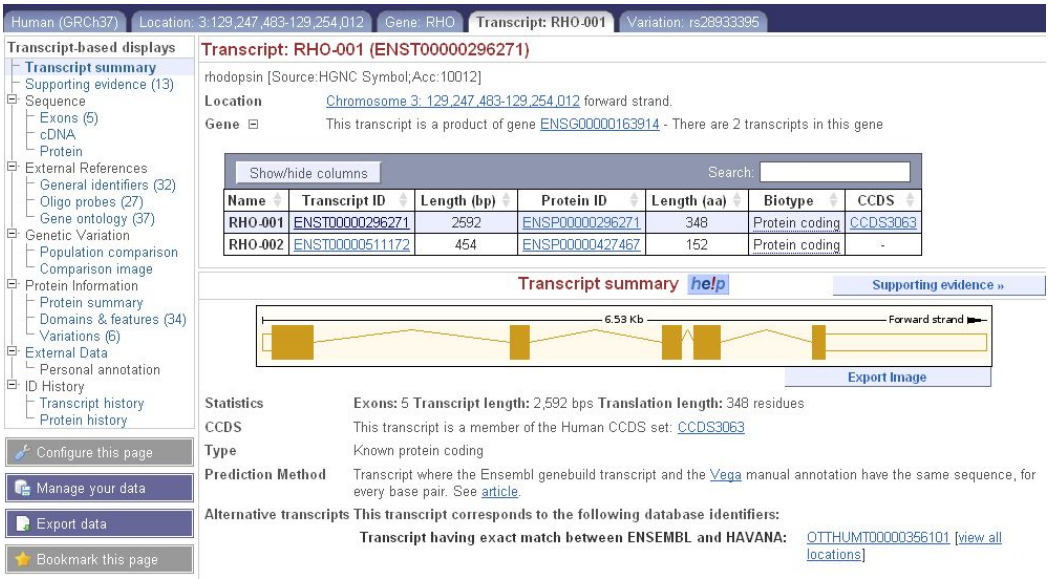
Then click on the *Variation image* (at the left).



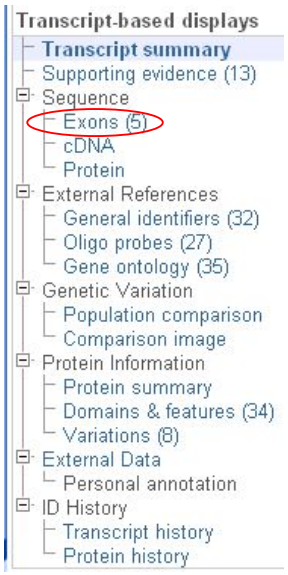
Click any variation, then *Variation properties* to learn more about it. A fourth tab will open:



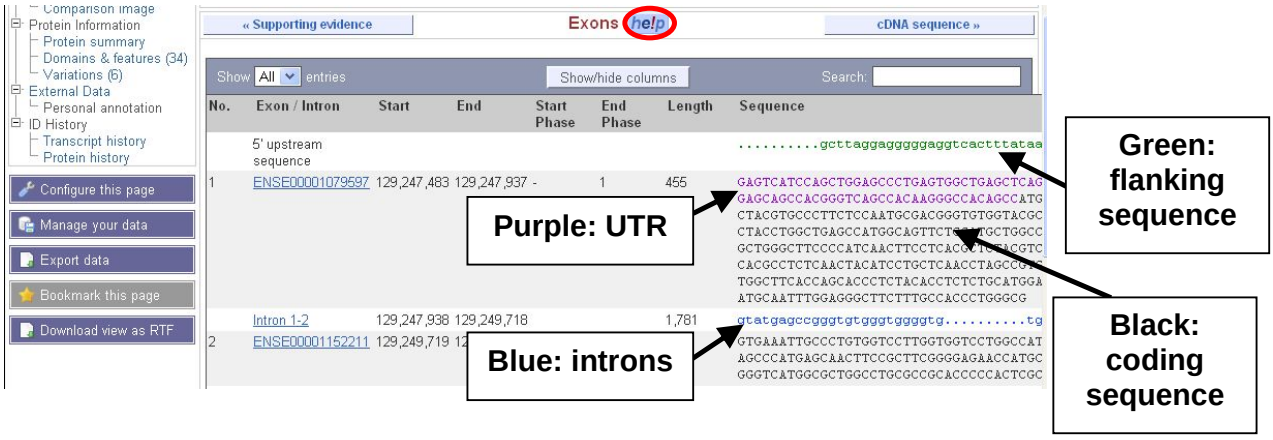
Go back to the *Gene tab*. Now, let's focus more closely on a transcript (spliced mRNA). Select the longer transcript from the table (ENST00000296271). This will lead to the Transcript summary display.



Again, the left hand navigation column provides several options for this particular transcript.



Choose the *Exons* option first, which highlights exon sequences. (exons, introns and flanking sequence are shown).

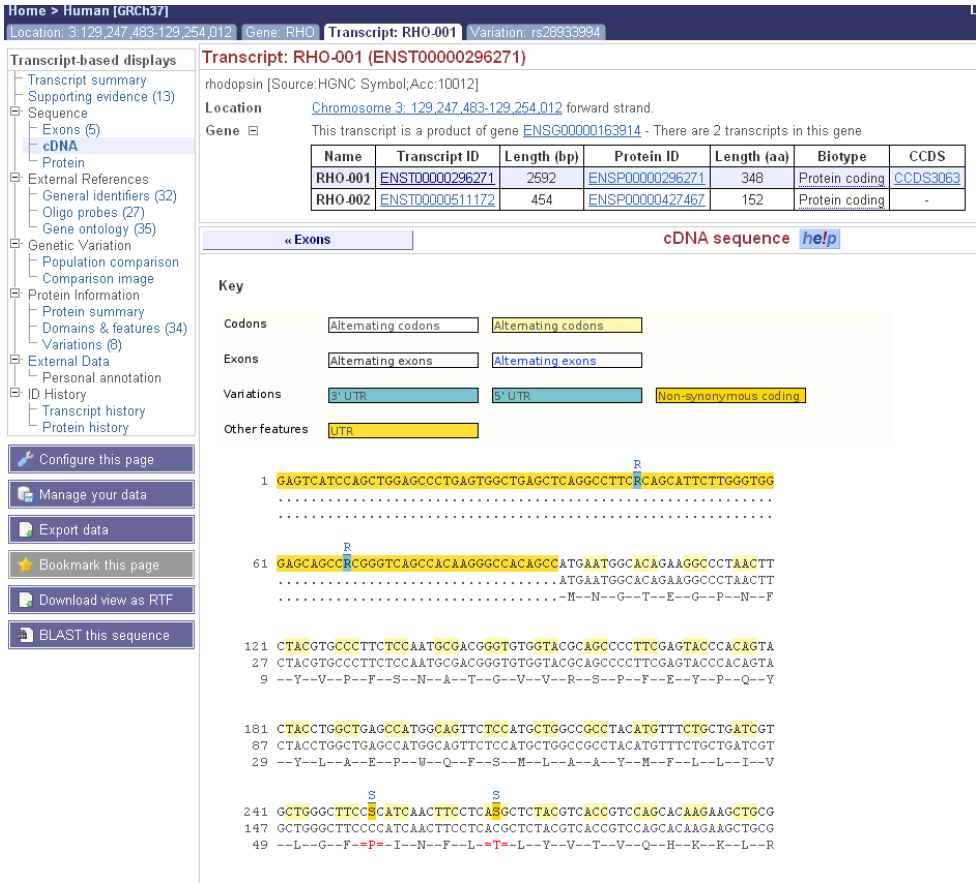


Click on the *Help* button (circled in red) for page specific help. A link to the glossary, FAQs, and videos is also provided.

You may use the 

link to change the display (for example, to show more flanking sequence, or to show full introns). If you would like to export this view, including the colours, click *Download view as RTF*. A "Rich Text Format" document will be generated that can be opened in Word.

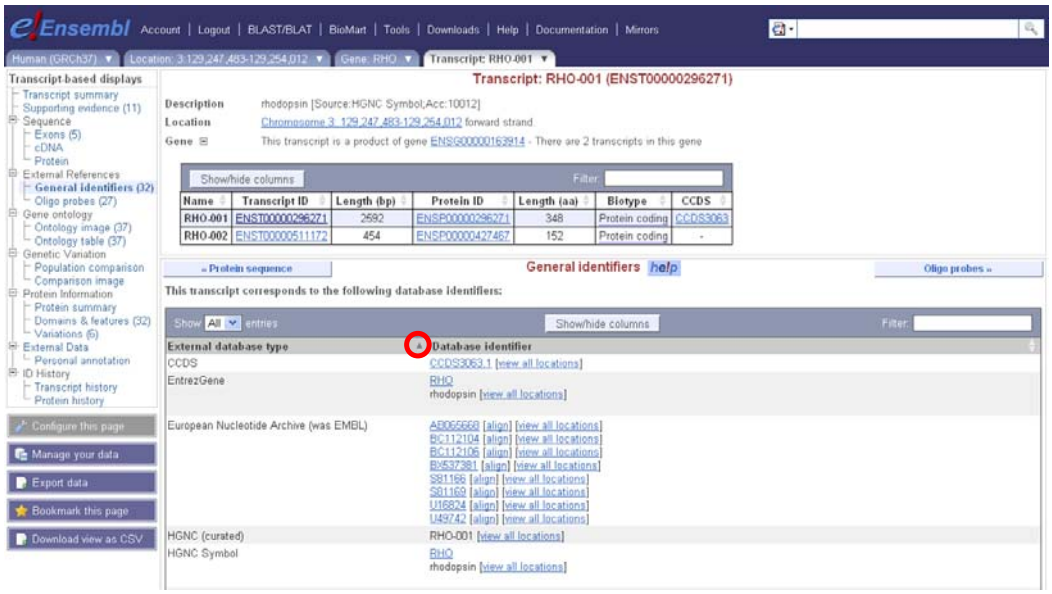
Now click the *cDNA* link to see the spliced transcript sequence.



UTR is highlighted in dark yellow, codons are highlighted in light yellow, and exon sequence is shown in black or blue letters to show exon divides.

Sequence variants are represented by highlighted nucleotides, and clickable IUPAC codes above the sequence.

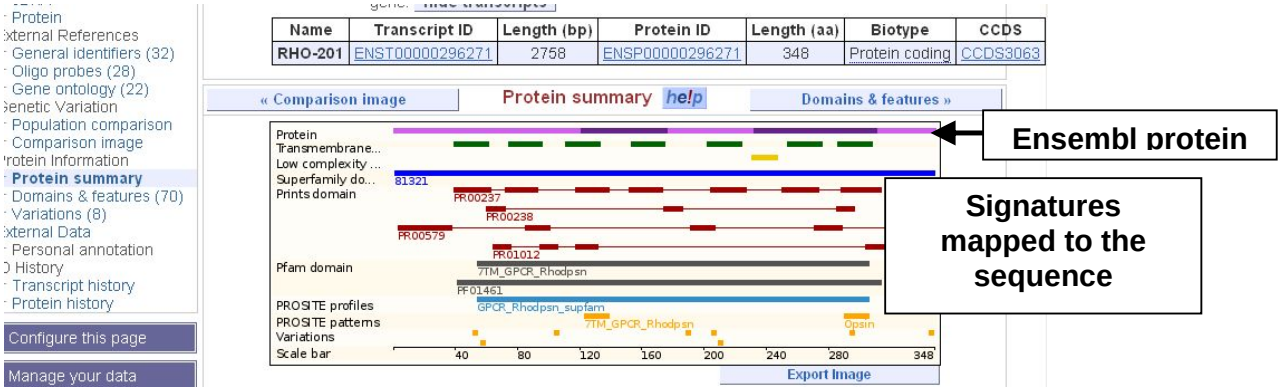
Next, follow the *General identifiers* link at the left, in the *External References* section.



This page can be ordered by "External database type" by clicking on the arrow (circled in red in the above figure) next to the title.

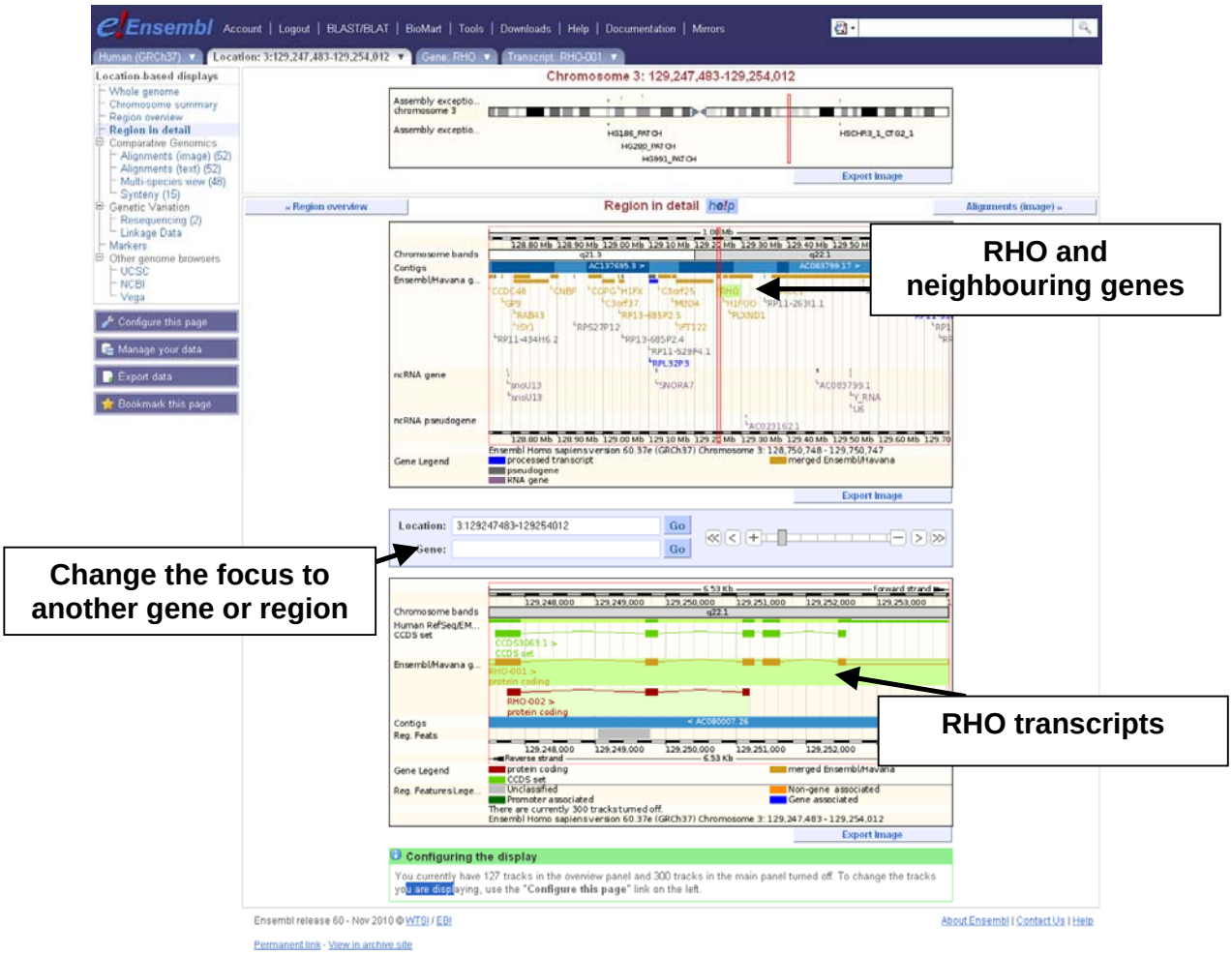
Other views include microarray probes and gene ontology terms from the GO consortium (*www.geneontology.org*).

Click on *Protein summary* to view mapped domains and signatures.



Clicking on *Domains & features* shows a table of protein signatures.

Let's now view the genomic region in which this gene and its transcript have been annotated by clicking onto the *Location* tab.

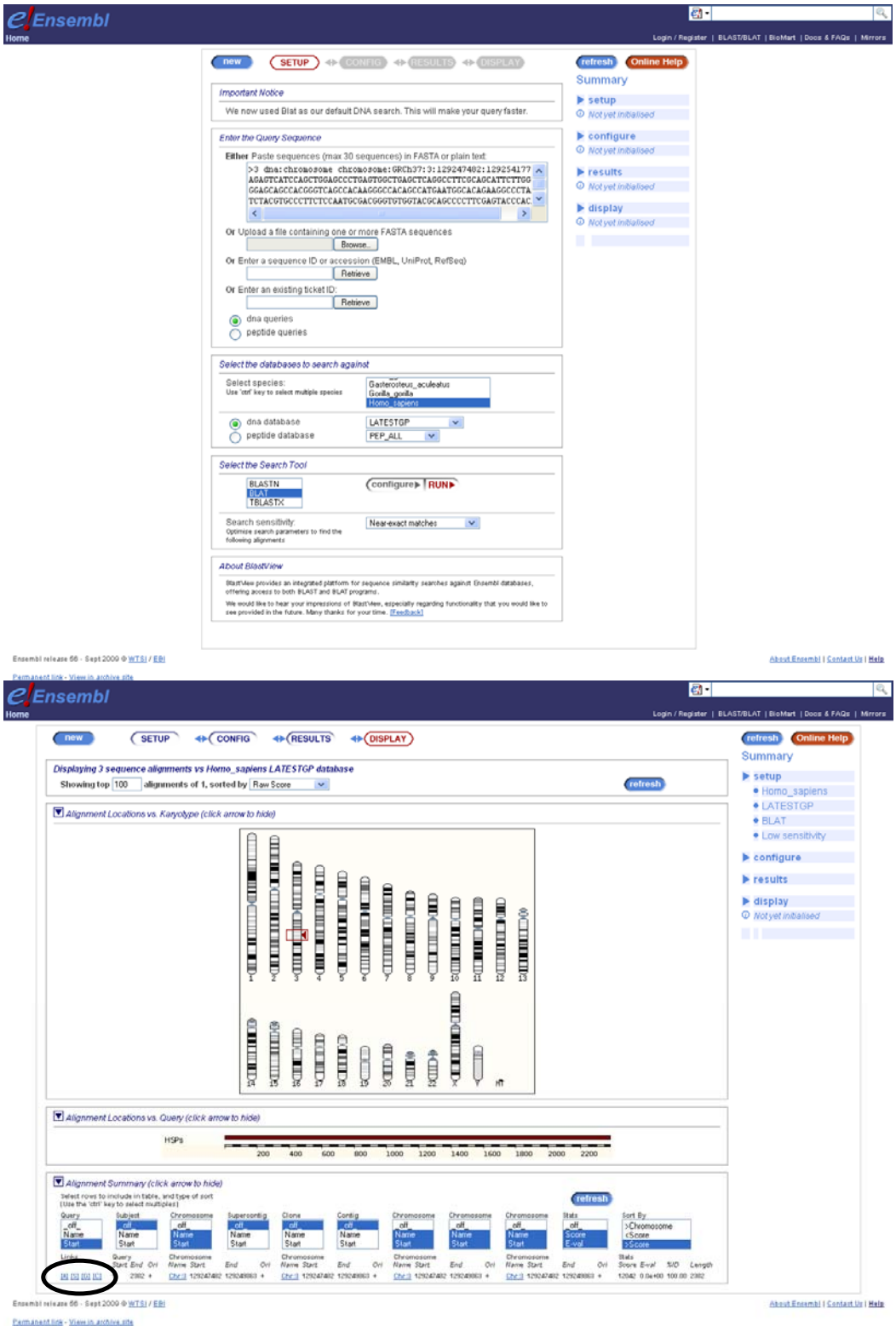


Ensembl *Location* displays are highly configurable. You can switch on additional tracks displaying various feature types that Ensembl annotates in the genome. Use the Configure this page **link to add *Sequence variants (all sources)* to the display. Also, choose to view the *Human UniProt prot.* track in normal, expanded form by choosing the *labels* option. Click on the "*Multiple alignments*" menu, and choose the three tracks for the *34 eutherian mammals* (including "*Conservation score*" and "*Constrained elements*"). Close the menu.**

After investigating the *Location display*, we would like to export genomic sequence. Click the *Export data* option and click *Next.*
Now click *HTML*.

```
>3 dna:chromosome chromosome:GRCh37:3:129247482:129254177:1
AGAGTCATCCAGCTGGAGCCCTGAGTGGCTGAGCTCAGGCCTTCGCAGCATTCTTGGGTG
GGAGCAGCCACGGGTCAGCCACAAGGGCCACAGCCATGAATGGCACAGAAGGCCCTAACT
TCTACGTGCCCTTCTCCAATGCGACGGGTGTGGTACGCAGCCCCTTCGAGTACCCACAGT
ACTACCTGGCTGAGCCATGGCAGTTCTCCATGCTGGCCGCCTACATGTTTCTGCTGATCG
TGCTGGGCTTCCCCATCAACTTCCTCACGCTCTACGTCACCGTCCAGCACAAGAAGCTGC
GCACGCCTCTCAACTACATCCTGCTCAACCTAGCCGTGGCTGACCTCTTCATGGTCCTAG
GTGGCTTCACCAGCACCCTCTACACCTCTCTGCATGGATACTTCGTCTTCGGGCCCACAG
GATGCAATTTGGAGGGCTTCTTTGCCACCCTGGGCGGTATGAGCCGGGTGTGGGTGGGGT
GTGCAGGAGCCCGGGAGCATGGAGGGGTCTGGGAGAGTCCCGGGCTTGGCGGTGGTGGCT
GAGAGGCCTTCTCCCTTCTCCTGTCCTGTCAATGTTATCCAAAGCCCTCATATATTCAGT
CAACAAACACCATTCATGGTGATAGCCGGGCTGCTGTTTGTGCAGGGCTGGCACTGAACA
CTGCCTTGATCTTATTTGGAGCAATATGCGCTTGTCTAATTTCACAGCAAGAAAACTGAG
CTGAGGCTCAAAGAAGTCAAGCGCCCTGCTGGGGCGTCACACAGGGACGGGTGCAGAGTT
GAGTTGGAAGCCCGCATCTATCTCGGGCCATGTTTGCAGCACCAAGCCTCTGTTTCCCTT
GGAGCAGCTGTGCTGAGTCAGACCCAGGCTGGGCACTGAGGGAGAGCTGGGCAAGCCAGA
CCCCTCCTCTCTGGGGGCCCAAGCTCAGGGTGGGAAGTGGATTTTCCATTCTCCAGTCAT
TGGGTCTTCCCTGTGCTGGGCAATGGGCTCGGTCCCCTCTGGCATCCTCTGCCTCCCCTC
TCAGCCCCTGTCCTCAGGTGCCCCTCCAGCCTCCCTGCCGCGTTCCAAGTCTCCTGGTGT
TGAGAACCGCAAGCAGCCGCTCTGAAGCAGTTCCTTTTTGCTTTAGAATAATGTCTTGCA
TTTAACAGGAAAACAGATGGGGTGCTGCAGGGATAACAGATCCCACTTAACAGAGAGGAA
AACTGAGGCAGGGAGAGGGGAAGAGACTCATTTAGGGATGTGGCCAGGCAGCAACAAGAG
CCTAGGTCTCCTGGCTGTGATCCAGGAATATCTCTGCTGAGATGCAGGAGGAGACGCTAG
```

Select the header and a few lines of sequence using Edit/Copy in your browser. Click on the *BLAST/BLAT* link in the bar at the top of the page. Paste the sequence into the appropriate box and select *BLAT* as the search algorithm. Finally, click *Run*.



Follow links (circled above) to an alignment [*A*], the query sequence [*S*], the genome sequence [*G*] and the corresponding Location View [*C*] (for its former name ContigView… or to C (see) the hit!).

Clicking on [*C*] should reveal the BLAT hit in "Region in detail":

You may click on the red bar for the score, %ID, and other BLAST/BLAT values.

III) EXERCISES and ANSWERS

Note: the answers to these exercises correspond to current version (60) of Ensembl. If you use these exercises in the future, after Ensembl is updated, please use the archive site for version 60.

BROWSING ENSEMBL

These exercises address using the browser to determine a variety of gene-relevant information such as transcript number and size, protein domains, functional classes and sequence.

Exercise 1 – Exploring a gene

(a) Find the human F9 (Coagulation factor IX Precursor) gene. On which chromosome and which strand of the genome is this gene located? How many transcripts (splice variants) are there? How many of these transcripts are protein coding?

(b) What is the longest Ensembl transcript? How long is the protein it encodes? How many exons does it have? Are any of the exons completely or partially untranslated?

(c) Which transcripts have a CCDS record associated with it? What does that mean?

(d) Have a look at the external references for ENST00000218099. What is the function of F9?

(e) Are there probesets on microarray platforms that can be used to monitor ENST00000218099 expression?

(f) Does ENSP00000218099 show peptidase activity?

(g) Go to the Protein summary view, if you are not there already. Click on the peptidase signature in the Pfam track. Note the ID number. Now click on the peptidase signature in the SMART track. Follow the link to the InterPro record. Do you see the Pfam ID?

(h) How many non-synonymous SNPs have been discovered for the protein encoded by ENST00000218099?

(i) Is there a mouse orthologue predicted for the human F9 gene?

Exercise 2 – Exploring a region

(a) Go to the region from bp 52,600,000 to 53,300,000 on human

chromosome 4. What does the transparent part in the 'Contigs' track represent? (If you are not sure what contigs are, click on Help and then Glossary.)

(b) Zoom in on the SGCB transcript, including a bit of flanking sequence on both sides.

(c) Is there a protein from UniProtKB that aligns to the genome at the same location as SGCB?

(d) Export the genomic sequence of the region you are looking at in FASTA format.

Answers (Browsing Ensembl)

1) (a) Go to the Ensembl homepage (http://www.ensembl.org).
Select '**Search: Human**' and type '**F9 gene**'
Click [Go].
Click on '**Homo sapiens**' on the page with search results.
Click on '**Gene**'.
Click on '**Ensembl protein_coding Gene: ENSG00000101981 (HGNC Symbol: F9)**'.

The human F9 gene is located on the X chromosome on the forward strand. Ensembl has three transcripts annotated for this gene, ENST00000218099 (F9-001), ENST00000394090 (F9-201), and ENST00000479617 (F9-002). Only F9-001 and F9-201 are protein coding.

(b) Look at the table. The longest transcript is ENST00000218099. The length of this transcript is 2780 base pairs and the length of the encoded protein is 461 amino acids. To see the exons, click on **ENST00000218099**. It has eight exons. Click on 'Sequence - Exons' in the side menu.
The first and last exon are partially untranslated (UTR sequence shown in purple).

Click "Show/hide columns" at the top to deselect columns you don't want (for example, select only "Sequence" to show the sequence alone).

(c) In the table of transcripts, there is a CCDS ID for F9-001 and F9-201. CCDS is the consensus coding sequence set. These coding sequences (CDS) have been agreed upon by Ensembl and NCBI. Since these two transcripts have the same CDS, they differ in the UTR alone.

(d) Click on '**External References - General identifiers**' in the side menu. Explore some of the links (good places to start are usually 'WikiGenes' and 'UniProtKB/Swiss-Prot').

Do the same for '**Gene ontology – Ontology table**'.

Factor IX is a vitamin K-dependent plasma protein that participates in the intrinsic pathway of blood coagulation by converting factor X to its active form in the presence of Ca^{2+} ions, phospholipids, and factor VIIIa (this is the description as found in UniProtKB/Swiss-Prot).

(e) Click on '**External References - Oligo probes**' in the side menu. Probesets from Affymetrix, Aglient, Codelink, Illumina, and Phalanx match to this transcript sequence.

(f) Click on 'ENST00000218099' if you are not already on the 'Transcript: F9-001' tab. Click on '**Protein Information – Protein summary**' in the side menu. And/or click on '**Protein Information - Domains & features**' in the side menu.

Alternatively, click on the link **ENSP00000218099** to jump to the **protein summary**. Peptidase signatures are found in the protein sequence, indicating there may be peptidase activity. Click on the "Peptidase" bars to see that the signature covers a range of amino acids (the different numbers reflect different lengths of the Peptidase signature in different databases, i.e. Superfamily, SMART, Pfam and PROSITE).

(g) The Pfam ID for the Peptidase_S1_S6 signature is PF00089. The InterPro ID is IPR001254. InterPro integrates information from different databases. The SMART, PROSITE, and Pfam signatures have been clustered into one InterPro record. PF00089 is listed in the "Signatures" section of the IPR001254 record. This can also be seen in the "Domains and features" table.

(h) Click on '**Protein Information - Variations**' in the side menu.
For the protein encoded by ENST00000218099 three non-synonymous SNPs have been discovered: rs6048, rs1801202 and rs4149751.

(i) Click on the '**Gene: F9**' tab.
Click on '**Orthologues'** in the side menu.
There is one mouse orthologue predicted for human F9, ENSMUSG00000031138.

Answer

2(a) Go to the Ensembl homepage.
Type **'human 4:52600000..53300000**' in the '**Search**' box.
Click **[Go]**.

The open part in the 'Contigs' track represents a gap in the genome assembly (although the human genome is called 'finished', there are still gaps!). Note that this region is very close to the centromere of the chromosome.

(b) Search for SGCB in the Gene box of the main panel. Zoom out one step in the zoom slide. Alternatively, **draw a box with your mouse** around the SGCB transcript. Click on 'Jump to region' in the pop-up menu.

(c) If it is not turned on, turn on the UniProt track as follows:
Click on '**Configure this page**' in the side menu.
Type '**UniProt**' in the 'Search display:' text box.
Select '**Human UniProt prot - Labels**.'
Close the menu.

NP_000223.1 aligns to the genome with the same exon structure as SGCB. Q16585 is another UniProt protein that aligns in the same place as one exon of SGCB. Click on it to see it is Beta-sarcoglycan.

(d) Click on **'Export data'** in the side menu.
Click **[Next>]**.
Click on **'Text'.**
Note that the sequence has a header that provides information about the genome assembly (GRCh37), the chromosome, the start and end coordinates and the strand. For example:

>4 dna:chromosome chromosome:GRCh37:4:52883261-52908260:1

The next section (BioMart) will show you how to export sequence in a different way.

IV) BioMart

Mining data- worked example

The human gene encoding Glucose-6-phosphate dehydrogenase (G6PD) is located on chromosome X in cytogenetic band q28.

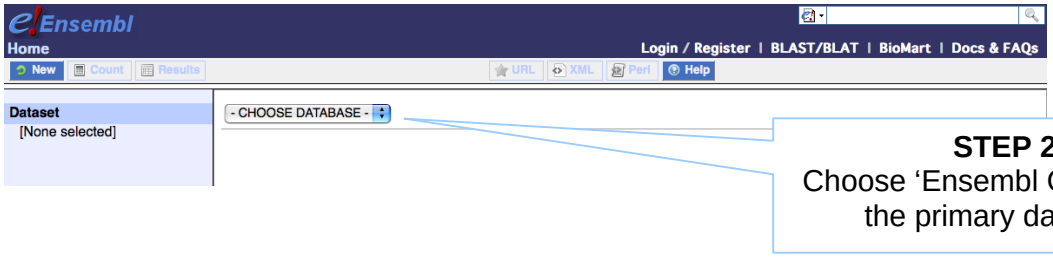
Which other genes with consensus coding sequences assigned by the CCDS project locate to the same band? What are their Ensembl Gene IDs and Entrez Gene IDs?

What are their cDNA sequences?

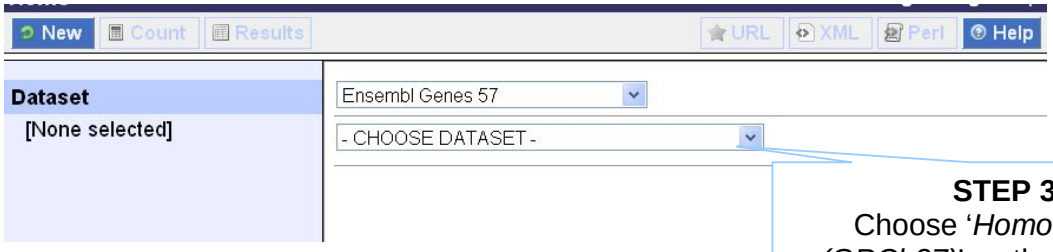
Follow the worked example below to answer these questions.

Step 1: Either click on 'BioMart' in the top header of an Ensembl page, or go to http://www.biomart.org/ and click on the 'MartView' tab.

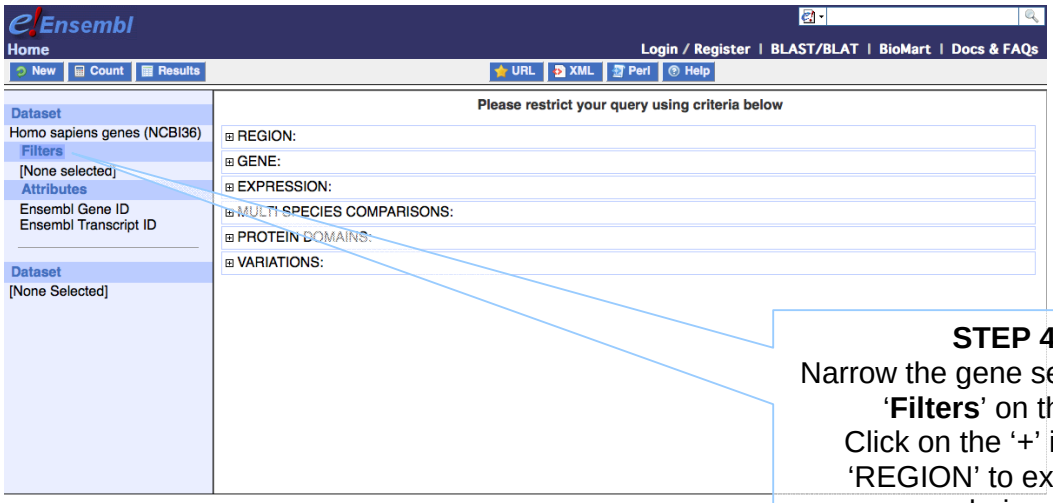
NOTE: These answers were determined using Ensembl 60



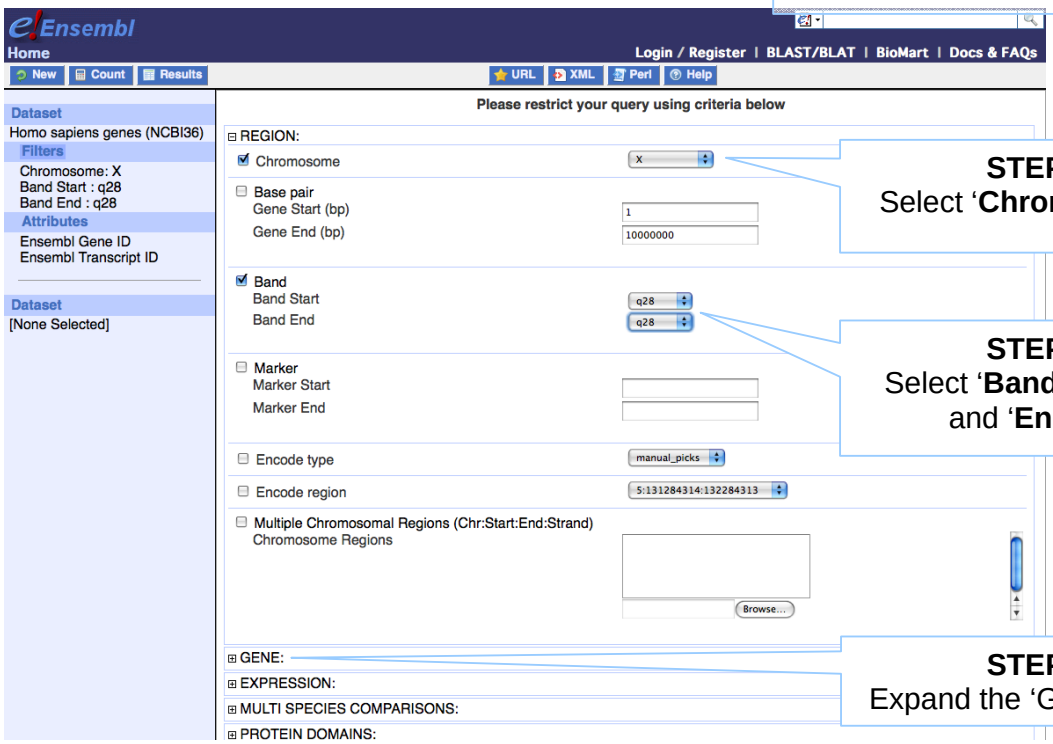
STEP 2:
Choose 'Ensembl Genes 60 as the primary database.



STEP 3:
Choose '*Homo sapiens (GRCh37)*' as the species of interest.



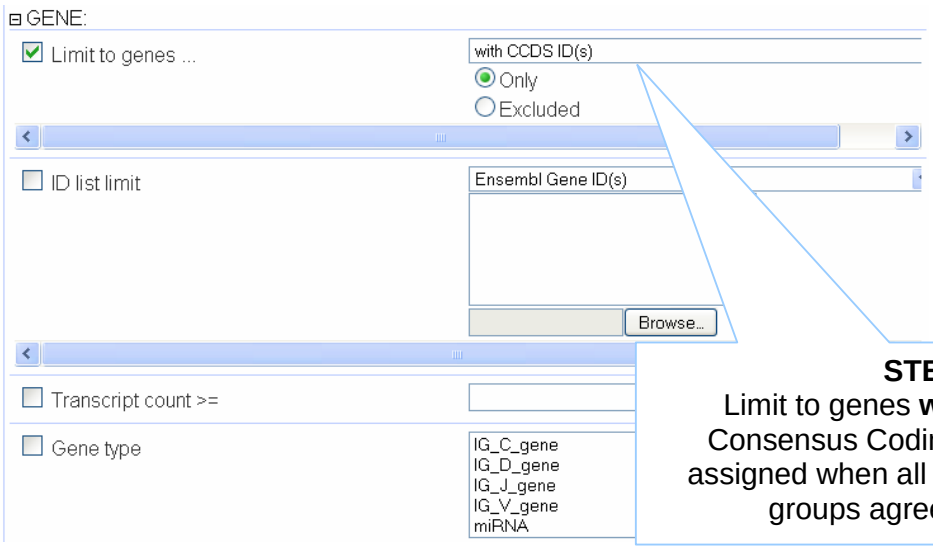
STEP 4:
Narrow the gene set by clicking '**Filters**' on the left.
Click on the '+' in front of 'REGION' to expand the choices.



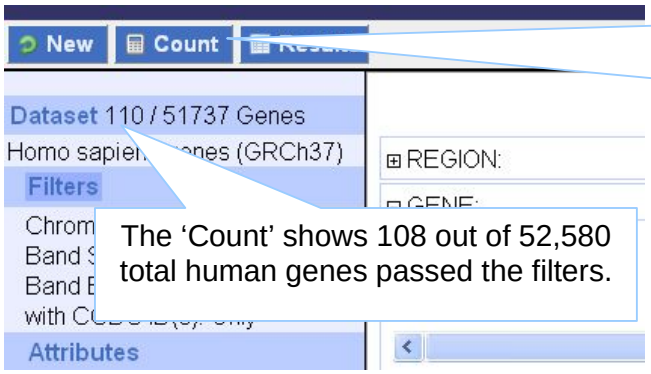
STEP 5:
Select '**Chromosome X**'

STEP 6:
Select '**Band Start q28**' and '**End q28**'

STEP 7:
Expand the 'GENE' panel.

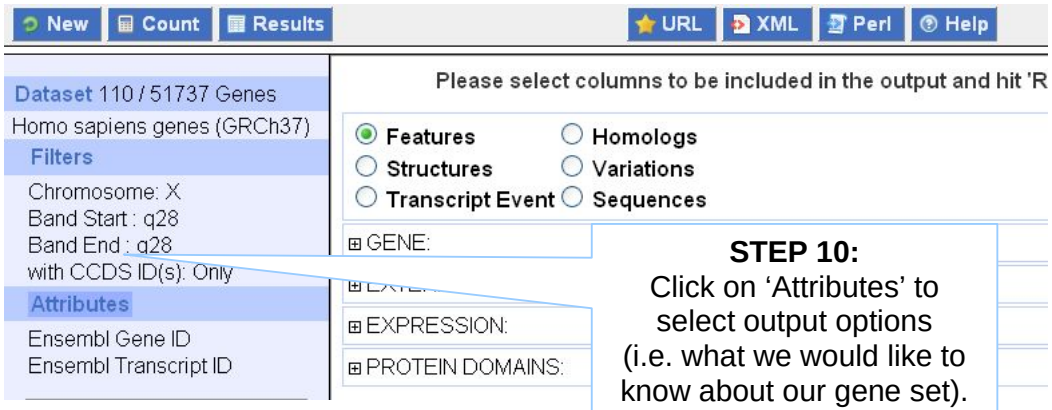


STEP 8:
Limit to genes **with CCDS ID(s)**. Consensus Coding Sequences are assigned when all genome annotation groups agree on a model.

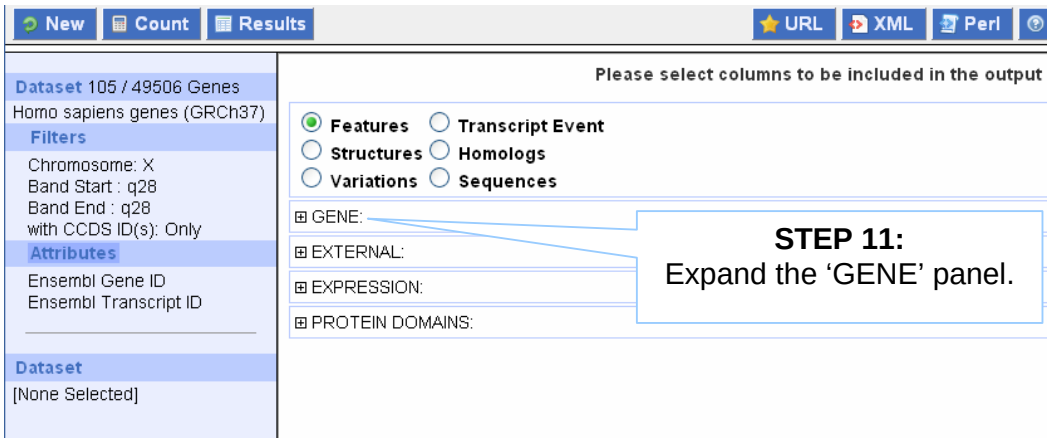


STEP 9:
The filters have determined our gene set.
Click **'Count'** to see how many genes have passed these filters.

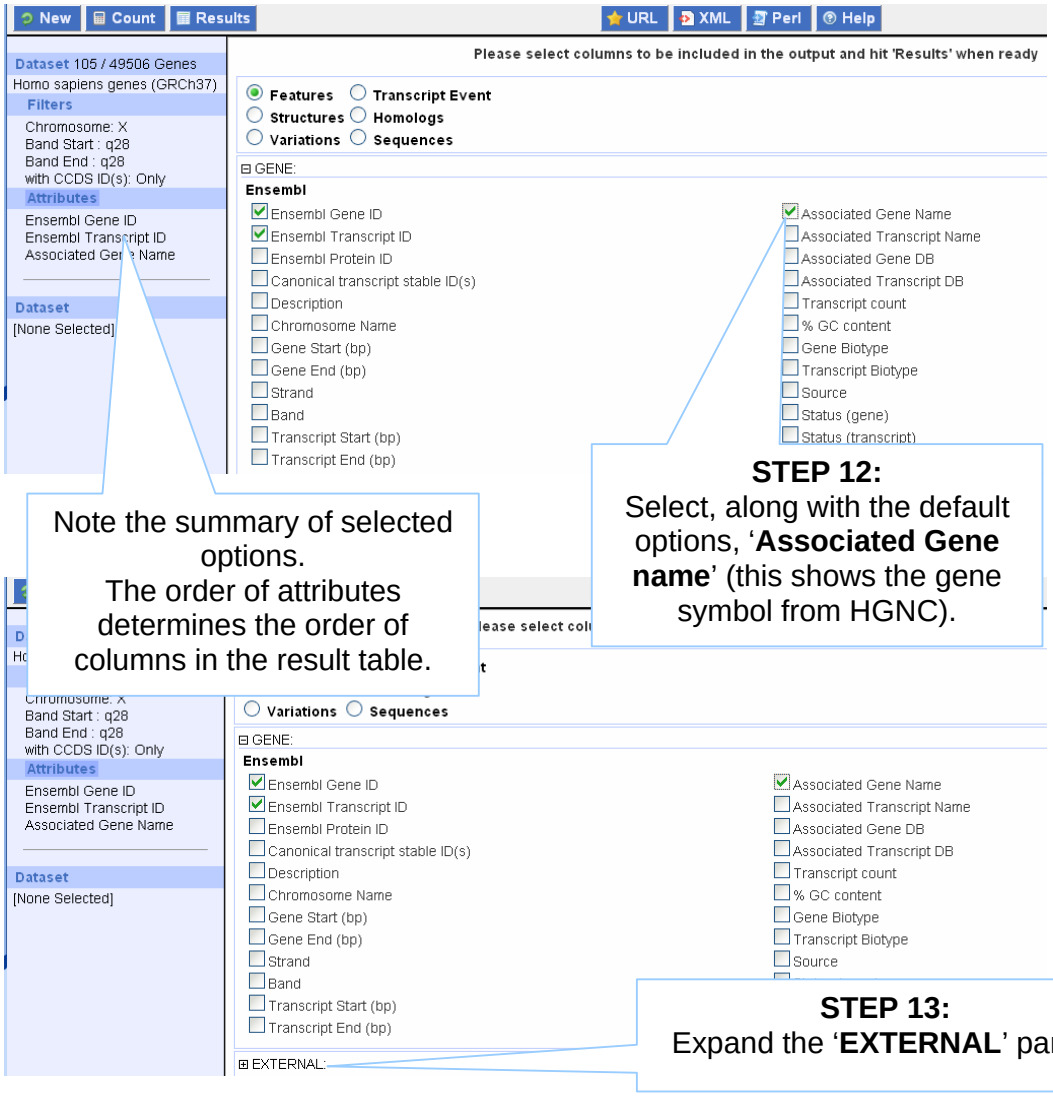
The 'Count' shows 108 out of 52,580 total human genes passed the filters.



STEP 10:
Click on 'Attributes' to select output options (i.e. what we would like to know about our gene set).



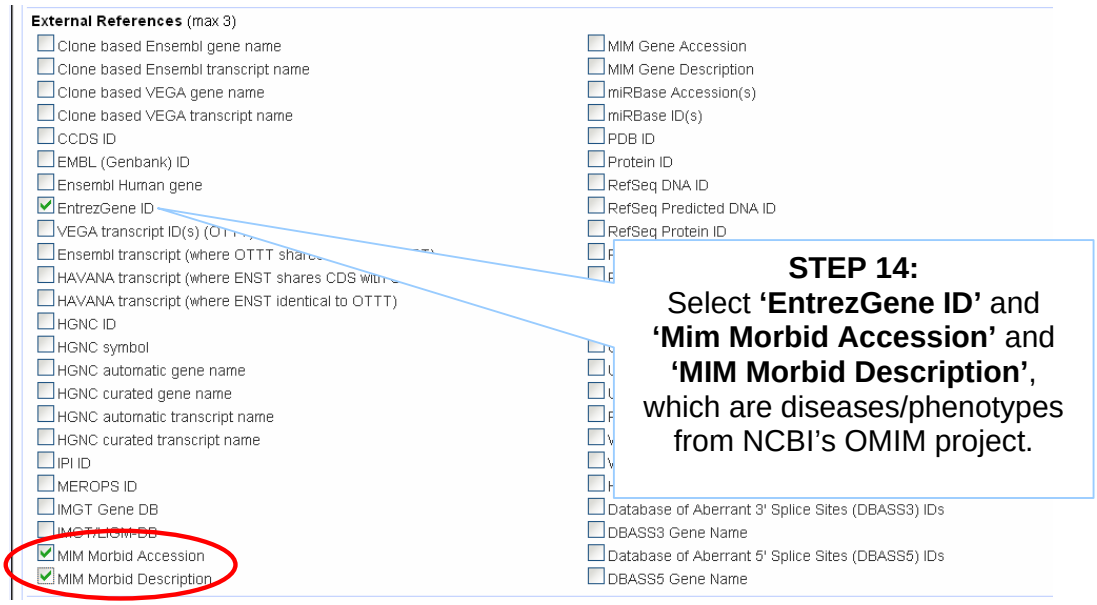
STEP 11:
Expand the 'GENE' panel.

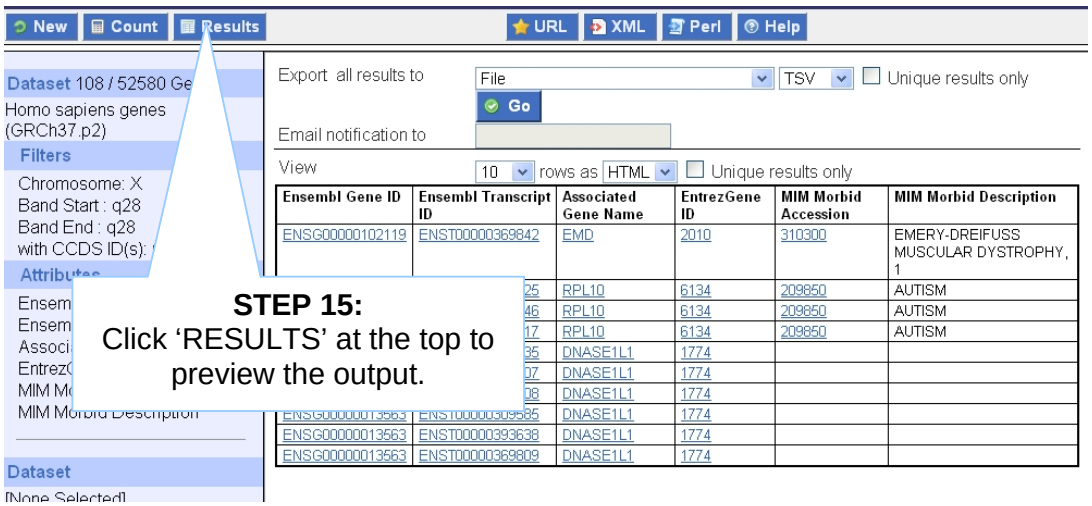


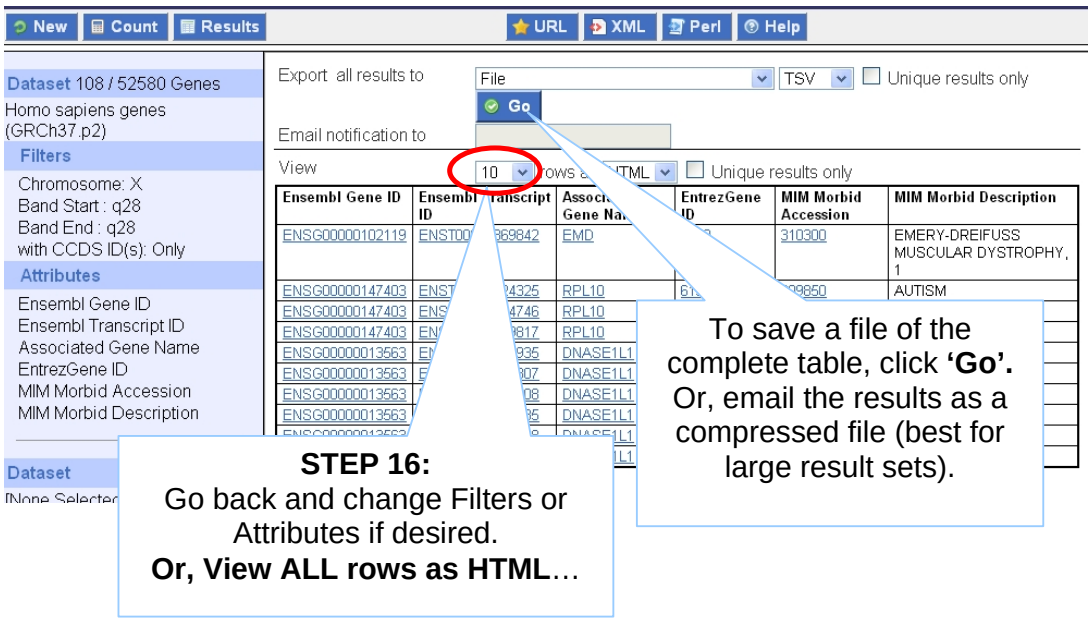
Note the summary of selected options.
The order of attributes determines the order of columns in the result table.

STEP 12:
Select, along with the default options, '**Associated Gene name**' (this shows the gene symbol from HGNC).

STEP 13:
Expand the '**EXTERNAL**' panel.

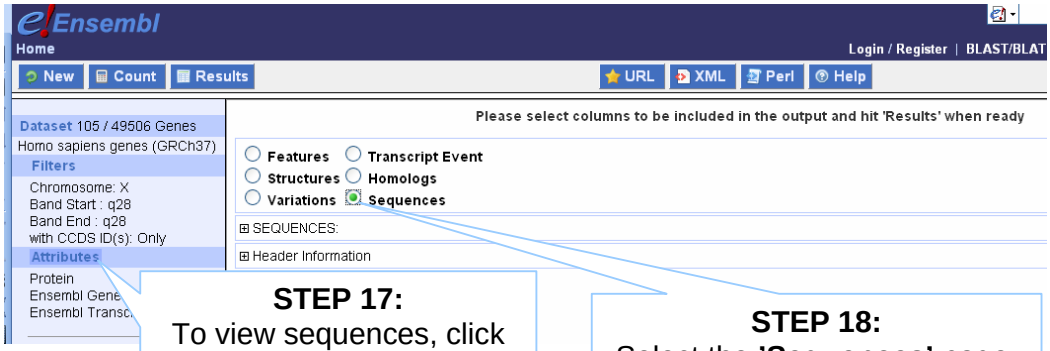

External References (max 3)
Clone based Ensembl gene name
Clone based Ensembl transcript name
Clone based VEGA gene name
Clone based VEGA transcript name
CCDS ID
EMBL (Genbank) ID
Ensembl Human gene
EntrezGene ID
VEGA transcript ID(s)
Ensembl transcript (where OTTT shares
HAVANA transcript (where ENST shares CDS with
HAVANA transcript (where ENST identical to OTTT)
HGNC ID
HGNC symbol
HGNC automatic gene name
HGNC curated gene name
HGNC automatic transcript name
HGNC curated transcript name
IPI ID
MEROPS ID
IMGT Gene DB
IMGT/LIGM-DB
MIM Morbid Accession
MIM Morbid Description
MIM Gene Accession
MIM Gene Description
miRBase Accession(s)
miRBase ID(s)
PDB ID
Protein ID
RefSeq DNA ID
RefSeq Predicted DNA ID
RefSeq Protein ID
Database of Aberrant 3' Splice Sites (DBASS3) IDs
DBASS3 Gene Name
Database of Aberrant 5' Splice Sites (DBASS5) IDs
DBASS5 Gene Name
STEP 14:
Select 'EntrezGene ID' and 'Mim Morbid Accession' and 'MIM Morbid Description', which are diseases/phenotypes from NCBI's OMIM project.


New
Count
Results
URL
XML
Perl
Help
Dataset 108 / 52580 Ge
Homo sapiens genes (GRCh37.p2)
Filters
Chromosome: X
Band Start : q28
Band End : q28
with CCDS ID(s):
Attributes
Dataset
[None Selected]
Export all results to
File
TSV
Unique results only
Go
Email notification to
View
10
rows as
HTML
Unique results only
Ensembl Gene ID
Ensembl Transcript ID
Associated Gene Name
EntrezGene ID
MIM Morbid Accession
MIM Morbid Description
ENSG00000102119 ENST00000369842 EMD 2010 310300 EMERY-DREIFUSS MUSCULAR DYSTROPHY, 1
RPL10 6134 209850 AUTISM
DNASE1L1 1774
STEP 15:
Click 'RESULTS' at the top to preview the output.


New
Count
Results
URL
XML
Perl
Help
Dataset 108 / 52580 Genes
Homo sapiens genes (GRCh37.p2)
Filters
Chromosome: X
Band Start : q28
Band End : q28
with CCDS ID(s): Only
Attributes
Ensembl Gene ID
Ensembl Transcript ID
Associated Gene Name
EntrezGene ID
MIM Morbid Accession
MIM Morbid Description
Dataset
Export all results to
File
TSV
Unique results only
Go
Email notification to
View
10
Unique results only
ENSG00000102119 EMD 310300 EMERY-DREIFUSS MUSCULAR DYSTROPHY, 1
ENSG00000147403 RPL10 AUTISM
STEP 16:
Go back and change Filters or Attributes if desired.
Or, View ALL rows as HTML…
To save a file of the complete table, click 'Go'. Or, email the results as a compressed file (best for large result sets).

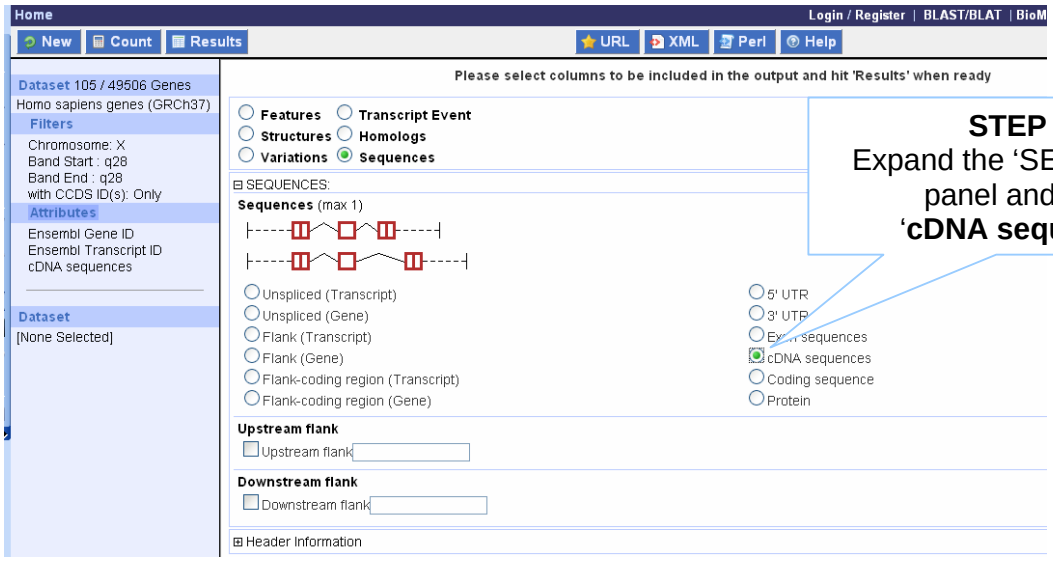
Ensembl Gene ID	Ensembl Transcript ID	Associated Gene Name	EntrezGene ID	MIM Morbid Accession	MIM Morbid Description
ENSG00000102119	ENST00000369842	EMD	2010	310300	EMERY-DREIFUSS MUSCULAR DYSTROPHY, 1
ENSG00000147403	ENST00000424325	RPL10	6134	209850	AUTISM
ENSG00000147403	ENST00000344746	RPL10	6134	209850	AUTISM
ENSG00000147403	ENST00000369817	RPL10	6134	209850	AUTISM
ENSG00000013563	ENST00000014935	DNASE1L1	1774		
ENSG00000013563	ENST00000369807	DNASE1L1	1774		
ENSG00000013563	ENST00000369808	DNASE1L1	1774		
ENSG00000013563	ENST00000309585	DNASE1L1	1774		
ENSG00000013563	ENST00000393638	DNASE1L1	1774		
ENSG00000013563	ENST00000369809	DNASE1L1	1774		
ENSG00000102125	ENST00000350743	TAZ	6901	300069	CARDIOMYOPATHY, DILATED, 3A
ENSG00000102125	ENST00000350743	TAZ	6901	300183	LEFT VENTRICULAR NONCOMPACTION, X-LINKED
ENSG00000102125	ENST00000350743	TAZ	6901	302060	BARTH SYNDROME
ENSG00000102125	ENST00000299328	TAZ	6901	300069	CARDIOMYOPATHY, DILATED, 3A
ENSG00000102125	ENST00000299328	TAZ	6901	300183	LEFT VENTRICULAR NONCOMPACTION, X-LINKED
ENSG00000102125	ENST00000299328	TAZ	6901	302060	BARTH SYNDROME
ENSG00000102125	ENST00000351413	TAZ	6901	300069	CARDIOMYOPATHY, DILATED, 3A

Result Table 1

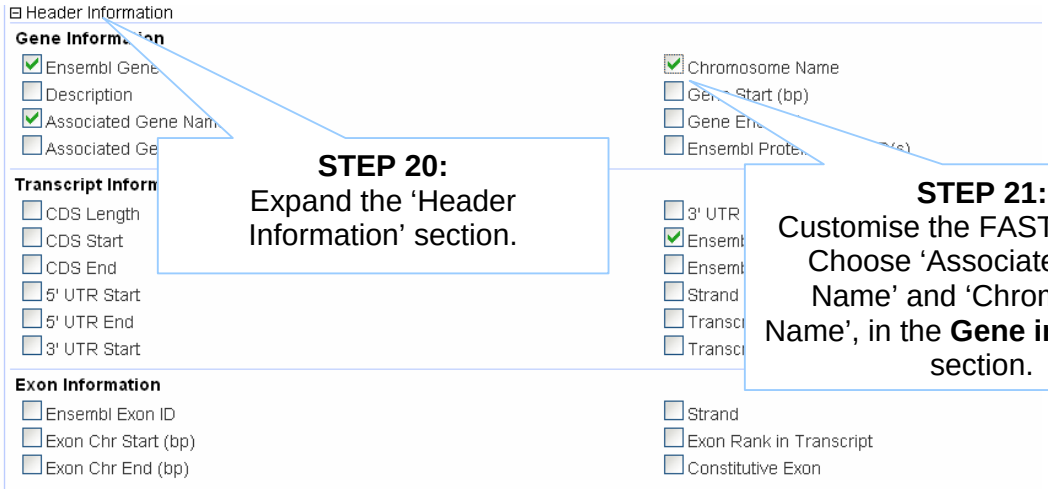


STEP 17:
To view sequences, click on **'Attributes'**

STEP 18:
Select the **'Sequences'** page, then expand the 'SEQUENCES' section.

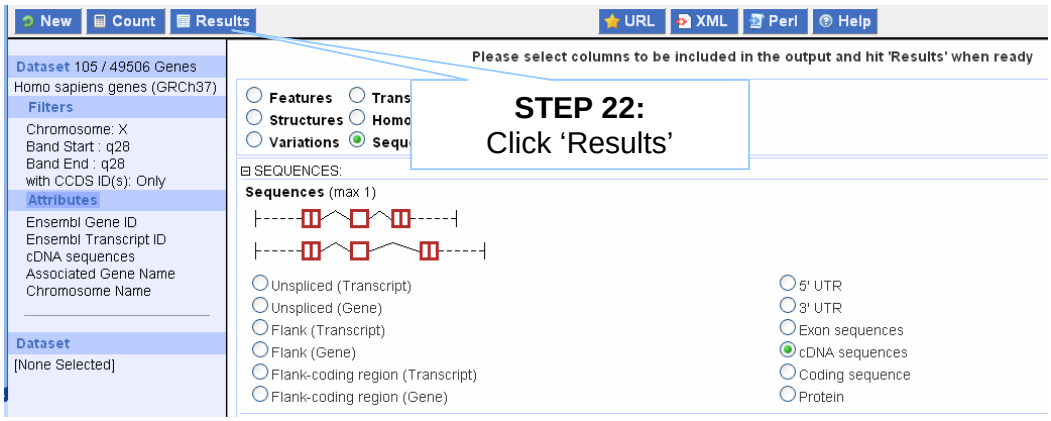


STEP 19:
Expand the 'SEQUENCES' panel and select '**cDNA sequences**'.

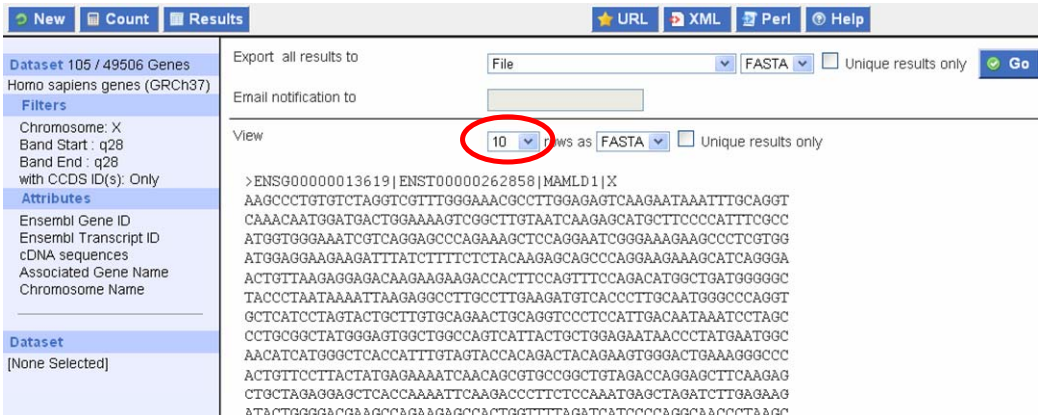


STEP 20:
Expand the 'Header Information' section.

STEP 21:
Customise the FASTA header. Choose 'Associated Gene Name' and 'Chromosome Name', in the **Gene information** section.



STEP 22:
Click 'Results'



Again, View ALL rows as FASTA for the full list… (make sure pop-up blocker is off):

>Header: Gene ID, Transcript ID, Gene Name, Chromosome

```
>ENSG00000013619|ENST00000262858|MAMLD1|X
AAGCCCTGTGTCTAGGTCGTTTGGGAAACGCCTTGGAGAGTCAAGAATAAATTTGCAGGT
CAAACAATGGATGACTGGAAAAGTCGGCTTGTAATCAAGAGCATGCTTCCCCATTTCGCC
ATGGTGGGAAATCGTCAGGAGCCCAGAAAGCTCCAGGAATCGGGAAAGAAGCCCTCGTGG
ATGGAGGAAGAAGATTTATCTTTTCTCTACAAGAGCAGCCCAGGAAGAAAGCATCAGGGA
ACTGTTAAGAGGAGACAAGAAGAAGACCACTTCCAGTTTCCAGACATGGCTGATGGGGGC
TACCCTAATAAAATTAAGAGGCCTTGCCTTGAAGATGTCACCCTTGCAATGGGCCCAGGT
GCTCATCCTAGTACTGCTTGTGCAGAACTGCAGGTCCCTCCATTGACAATAAATCCTAGC
CCTGCGGCTATGGGAGTGGCTGGCCAGTCATTACTGCTGGAGAATAACCCTATGAATGGC
AACATCATGGGCTCACCATTTGTAGTACCACAGACTACAGAAGTGGGACTGAAAGGGCCC
ACTGTTCCTTACTATGAGAAAATCAACAGCGTGCCGGCTGTAGACCAGGAGCTTCAAGAG
CTGCTAGAGGAGCTCACCAAAATTCAAGACCCTTCTCCAAATGAGCTAGATCTTGAGAAG
ATACTGGGGACGAAGCCAGAAGAGCCACTGGTTTTAGATCATCCCCAGGCAACCCTAAGC
ACAACTCCCAAGCCTTCGGTTCAGATGTCACACTTGGAGAGCCTGGCTTCCAGCAAGGAG
TTTGCTTCTAGTTGCAGCCAAGTTACTGGCATGTCACTTCAGATCCCATCCTCCTCCACA
.................
ATAGAAAACCCACACCCACTGTCCTGTAAACTTTTCTCAGTGTCCAGACTTTCTGTAATC
ACATTTTAATTGCCACCTCGTATTTCACCTCTACATTTGAAATCTGGCGTCTGTTTCAAG
CCAGTGTGTTTTTTCTTCGTTCTGTAATAAACAGCCAGGAGAAAAGTG
>ENSG00000166008|ENST00000298974|MAGEA9|X
GTGCGCACTGGGGGTCAGAGAGAAGGGAGAGGCCTCCTTCTGAGGGGCGGCTTGATACCG
GTGGAGGAGCTCCAGGAAGCAGGCAGGCCTTGGTCTGAGACAGTGTCCTCAGGTCGCAGA
GCAGAGGAGACCCAGGCAGTGTCAGCAGTGAAGGTTCTCGGGACAGGCTAACCAGGAGGA
CAGGAGCCCCAAGAGGCCCCAGAGCAGCACTGACGAAGACCTGCCTGTGGGTCTCCATCG
CCCAGCTCCTGCCCACGCTCCTGACTGCTGCCCTGACCAGAGTCATCATGTCTCTCGAGC
AGAGGAGTCCGCACTGCAAGCCTGATGAAGACCTTGAAGCCCAAGGAGAGGACTTGGGCC
TGATGGGTGCACAGGAACCCACAGGCGAGGAGGAGGAGACTACCTCCTCCTCTGACAGCA
AGGAGGAGGAGGTGTCTGCTGCTGGGTCATCAAGTCCTCCCCAGAGTCCTCAGGGAGGCG
CTTCCTCCTCCATTTCCGTCTACTACACTTTATGGAGCCAATTCGATGAGGGCTCCAGCA
GTCAAGAAGAGGAAGAGCCAAGCTCCTCGGTCGACCCAGCTCAGCTGGAGTTCATGTTCC
AAGAAGCACTGAAATTGAAGGTGGCTGAGTTGGTTCATTTCCTGCTCCACAAATATCGAG
TCAAGGAGCCGGTCACAAAGGCAGAAATGCTGGAGAGCGTCATCAAAAATTACAAGCGCT
```

cDNA 1

cDNA 2

V) BioMart Exercises and Answers

These exercises have been designed to familiarise you with different questions you can answer with this tool, and the types of data you can retrieve with BioMart.

1. Retrieve all SNPs for 'known' human G-protein coupled receptor genes (GPCRs – use the InterPro domain ID: IPR000276) on chromosome 2.

Note: As this is the first exercise we walk you this time through BioMart step-by-step (but of course you can also try to do this exercise without our help!)

Start a new BioMart session by clicking 'New', or go back to the Ensembl homepage and click on 'Mine Ensembl with BioMart' under 'Ensembl tools'.

Choose the **database** and the **dataset** for your query as follows:
- Select 'Ensembl Genes 60'
- Select 'Homo sapiens genes (GRCh37.p2)'.

Click on '**Filters**' at the left. Filter this dataset to select your genes of interest as follows:
- Expand the '**REGION**' section at the right by clicking on the '+'. Select '**Chromosome 2**'. Click [count] at the top of the panel and note the number of Ensembl genes on *Homo sapiens* chromosome 2.
- In the '**GENE**' section, select 'Status (gene)' '**KNOWN**'.
- In the '**PROTEIN DOMAINS**' section, select the 'Limit to genes with these family or domain IDs' option. Select '**InterPro ID(s)**' and enter '**IPR000276**' in the box. Click [count] again and note that the number of genes is now **25**.

Click on '**Attributes**' (at the left). Select the output for your gene list as follows:
- Select the '**Variations**' Attribute Page.
- In the '**GENE**' section 'Ensembl Gene ID' and 'Ensembl Transcript ID' are selected by default – also select '**Ensembl Protein ID**'.
- In the '**GERM LINE ASSOCIATED VARIATIONS**' section 'Reference ID' is selected. Also select '**Allele**', '**Protein location (aa)**', and '**Protein Allele**'.

Note: Clicking on count now will not show an altered number. Attribute selections should not affect the count (i.e. the number of genes that have passed the filters).

Click on '**Results**' (at the top) to obtain the first 10 rows of your table. To obtain the entire table select 'View all rows as HTML' or export a file by clicking 'Go'. Check the box 'Unique results only'; otherwise you can end up with redundant rows!!

Why are several columns in the preview table blank? Because not all variations are within the coding sequence.

Exercise 2

Generate a list of all zebrafish protein-coding genes that are located on chromosome 3. Export gene name, description, Zfin symbol, and InterPro domains.

Exercise 3

For this exercise, it's easier to cut and paste the IDs from the online course booklet. One copy is here:

http://www.ebi.ac.uk/~gspudich/workshop_presentations/CNIO_nov2010/coursebook_60_v.pdf

BioMart is a very handy tool when you want to convert IDs from different databases. The following is a list of 29 IDs of human proteins from the RefSeq database of NCBI (http://www.ncbi.nlm.nih.gov/projects/RefSeq/):

NP_001218, NP_203125, NP_203124, NP_203126, NP_001007233, NP_150636, NP_150635, NP_001214, NP_150637, NP_150634, NP_150649, NP_001216, NP_116787, NP_001217, NP_127463, NP_001220, NP_004338, NP_004337, NP_116786, NP_036246, NP_116756, NP_116759, NP_001221, NP_203519, NP_001073594, NP_001219, NP_001073593, NP_203520, NP_203522

Generate a list that shows to which Ensembl Gene IDs and to which HGNC symbols these RefSeq IDs correspond.

Exercise 4

In a paper from 1995 Ayyagari *et al.* mapped the human 'Usher Syndrome type I C' to the genomic region between the markers D11S1397 and D11S1310 (Mol. Vis. 1:2, 1995).

Confirm this finding by generating a list of the genes located in this region.

Exercise 5

Forrest *et al.* performed a microarray analysis of peripheral blood mononuclear cell gene expression in benzene-exposed workers (Environ Health Perspect. 2005 June; 113(6): 801–807). The microarray used was the Affymetrix U133A/B (also called U133 plus 2) GeneChip. The top 25 up-regulated probe-sets were:

207630_s_at, 221840_at, 219228_at, 204924_at 227613_at, 223454_at, 228962_at, 214696_at, 210732_s_at, 212371_at, 225390_s_at, 227645_at, 226652_at, 221641_s_at, 202055_at, 226743_at, 228393_s_at, 225120_at, 218515_at, 202224_at, 200614_at, 212014_x_at, 223461_at, 209835_x_at, 213315_x_at

(a) Retrieve for the genes corresponding to these probe-sets the Ensembl Gene and Transcript IDs as well as their HGNC symbols (as far as available) and descriptions.

(b) In order to analyse these genes for possible promoter/enhancer elements, retrieve the 2000 bp upstream of the transcripts of these genes.

(c) In order to be able to study these human genes in mouse, identify their mouse orthologues. Also retrieve the genomic coordinates of these orthologues.

Exercise 6

Known dolphin genes match to a protein or mRNA sequence in a public database for dolphin (this is in contrast to 'known by projection' which was based on evidence from another species).

Step 1: For all known dolphin genes in Ensembl, export human homologues.

Step 2: ***Advanced:*** export a list of the human gene IDs alone (select only one attribute, and then select 'Unique results only'.) Do a second query in BioMart with human genes, upload these gene IDs and export gene names!

Exercise 7

List all variations on human chromosome 1 between nucleotides 800,000 and 850,000. Export genes associated with these variations (if any).

Answers: BIOMART

1. You should find ***25*** known genes on chromosome 2 with this InterPro domain. The result table is quite large; so don't export the entire table if export is going slowly.

2. Click '**New**' for a new query. **HINT**, if BioMart is not clearing, click on the "**BioMart**" link at the top of the page rather than "New".

Start with all the zebrafish Ensembl genes:

Choose the '**ENSEMBL Genes 60**' database.
Choose the '***Danio rerio genes (Zv9)***' dataset.

Now filter for the genes on chromosome 3:

Click on **'Filters**' in the left panel.
Expand the '**REGION**' section by clicking on the + box.
Select '**Chromosome 3**'. Make sure the check box in front of the filter is ticked otherwise the filter won't work.

Now filter further for genes that are protein coding:

Expand the '**GENE**' section by clicking on the + box.
Select '**Gene type'** as **'protein_coding**'.
Click the [Count] button on the toolbar.

This should give you 1268 / 28491 Genes.

Specify the attributes to be included in the output (note that a number of attributes will already be default selected):

Click on '**Attributes**' in the left panel.
Select the '**Features**' attributes page.
Expand the '**GENE**' section by clicking on the + box.
Select, in addition to the attributes 'Ensembl Gene ID' and 'Ensembl Transcript ID' that are already default selected, '**Associated Gene Name**' and '**Description**'.

Expand the '**EXTERNAL**' panel to select **ZFIN symbols**. These will be equal to the Gene Name, when available.

Expand the '**PROTEIN DOMAINS**' section to add '**InterPro ID**' '**InterPro Short Description**'.

Click the [**Results**] button on the toolbar.

Select '**View All rows as HTML**' or export all results to a file.

3. Click [New].
Choose the '**ENSEMBL Genes 60**' database.
Choose the '***Homo sapiens genes (GRCh37)'*** dataset.

Click on **'Filters'** in the left panel.
Expand the '**GENE'** section by clicking on the + box.
Select **'ID list limit - RefSeq protein ID(s)'** and enter the list of IDs in the text box (either comma separated or as a list).
HINT: You may have to scroll down the menu to see these.

Click on **'Attributes'** in the left panel.
Select the **'Features'** attributes page.
Expand the **'GENE'** section by clicking on the + box.
Deselect 'Ensembl Transcript ID'.
Expand the **'External'** section by clicking on the + box.
Select '**HGNC symbol'** and '**RefSeq Protein ID' f**rom the **'External References'** section.

Click the [Results] button on the toolbar.
Select 'View All rows as HTML' or export all results to a file. Tick the box 'Unique results only'.

Note: BioMart is 'transcript-centric', which means that it will give a separate row of output for each transcript of a gene, even if you don't include the Ensembl Transcript ID in your output. When you don't want this, use the 'Unique results only' option.

Your results should show that the RefSeq IDs map to **10** genes (you can also see this by clicking 'Count').

4. Click **[New].**
Choose the **'ENSEMBL Genes 60'** database.
Choose the '***Homo sapiens genes*** (GRCh37)' dataset.

Click on **'Filters'** in the left panel.
Expand the **'REGION'** section by clicking on the + box.
Enter 'Marker Start: **D11S1397**' and 'Marker End: **D11S1310**'.

Click on **'Attributes'** in the left panel.
Select the **'Features'** attributes page.
Expand the **'GENE'** section by clicking on the + box.
Deselect 'Ensembl Transcript ID'.
Select **'Associated Gene Name'** and **'Description'**.

Click the [Results] button on the toolbar.
Select 'View All rows as HTML' or export all results to a file. Tick the box 'Unique results only'.

Your results should show **31** genes. There should be one gene (ENSG00000006611) with the name 'USH1C' and description 'Harmonin (Usher syndrome type-1C protein) (Autoimmune enteropathy-related antigen AIE-75) (Antigen NY-CO-38/NY-CO-37) (PDZ-73 protein) (Renal carcinoma antigen NY-REN-3). [Source:UniprotKB/SWISSPROT;Acc:Q9Y6N9]'. This suggests that Ayyagari et al. correctly mapped Usher Syndrome type I C to this genomic region. The gene may not be in the first 10 rows, so view more rows to see it.

5. (a) Click **[New].**
Choose the **'ENSEMBL Genes 60'** database.
Choose the 'Homo sapiens genes (GRCh37)' dataset.

Click on **'Filters'** in the left panel.
Expand the '**GENE'** section by clicking on the + box.
Select **'ID list limit - Affy hg u133 plus 2 ID(s)'** and enter the list of probe-set IDs in the text box (either comma separated or as a list).

Click on **'Attributes'** in the left panel.
Select the **'Features'** attributes page.
Expand the **'GENE'** section by clicking on the + box.
Select, in addition to the default selected attributes, '**Description**'.
Expand the '**External'** section by clicking on the + box.
Select **'HGNC symbol'** from the '**External References**' section and '**AFFY HG U133-PLUS-2**' from the '**Microarray Attributes**' section.

Click the **[Results]** button on the toolbar.
Select 'View All rows as HTML' or export all results to a file. Tick the box 'Unique results only'.

Your results should show that the 25 probes map to 23 Ensembl genes.

(b) Don't change Dataset and Filters- simply click on '**Attributes**'.

Select the **'Sequences'** attributes page.
Expand the **'SEQUENCES'** section by clicking on the + box.
Select **'Flank (Transcript)'** and enter **'2000'** in the **'Upstream flank'** text box.
Expand the **'Header information'** section by clicking on the + box.
Select, in addition to the default selected attributes, **'Description'** and **'Associated Gene Name'.**

Note: 'Flank (Transcript)' will give the flanks for all transcripts of a gene with multiple transcripts. 'Flank (Gene)' will give the flanks for the transcript with the outermost 5' or 3' end.

Click the **[Results]** button on the toolbar.

(c) You can leave the Dataset and Filters the same, and go directly to the **'Attributes'** section:

Click on '**Attributes'** in the left panel.
Select the **'Homologs'** attributes page.
Expand the **'GENE'** section by clicking on the + box.
Select **'Associated Gene Name'.**
Deselect 'Ensembl Transcript ID'.
Expand the **'MOUSE ORTHOLOGS'** section by clicking on the + box.
Select '**Mouse Ensembl Gene ID'**, **'Mouse Chromosome', 'Mouse Chr Start (bp)**' and **'Mouse Chr End (bp)'.**

Click the [Results] button on the toolbar.
Check the box 'Unique results only'. Select 'View All rows as HTML' or export all results to a file.

Your results should show that for **21** out of the 23 human genes at least one mouse orthologue has been identified. ENSG00000123130 has two mouse orthologues and ENSG00000172716 has three. Two human genes (ENSG00000186594 and ENSG00000130844) have none.

6. Step 1: Choose *'Ensembl Genes 60'* and *'Tursiops truncatus genes (turTru1)'*. Filters: Expand the *'GENE'* panel and select Status (gene) as *'KNOWN'*.
Attributes: Select *'Human Ensembl Gene ID'* under the *'Homologs'* page.

Step 2: Remove *'Ensembl Gene ID'*, *'Ensembl Transcript ID'*, and *'Ensembl Protein ID'* from the Attributes. Click on *'Unique results only'* and export the file.
Click NEW. Start with *'Ensembl 60 genes'*, '*Homo sapiens genes (GRCh37)'*.
Filters: Expand the GENE panel, and click browse to upload a file into the *'ID List Limit Box'*.
In Attributes, select 'Gene Name'.
Click Results.

7. In this exercise, select "Ensembl Variation 60" as the Database, and "Homo sapiens Variation" as the Dataset. This allows intergenic variations to be chosen. You would also use the Variation Mart if you had a list of variation IDs to input in the filters.

Filter with REGION: Chromosome 1
Base pair start: 800000
end: 850000

Click 'Count'. Note this counts variations, not genes. You should see 770.

To the **Attributes**, add "Ensembl Gene ID", "Ensembl Transcript ID", and "Consequence to transcript" under the GENE ASSOCIATED INFORMATION panel.

VI) EXERCISES COMPARATIVE GENOMICS

Exercise 1 - Orthologues, paralogues and genetrees

Find the human Ensembl SMAD2 gene.

(a) How many within-species paralogues are predicted for this gene?
Note the Target %id and Query %id. Which paralogue has the most sequence similarity with SMAD2?

Retrieve an alignment between SMAD2 and one of its paralogues.

(b) Is there an orthologue predicted for this gene in gorilla?

(c) Have a look at the genetree for this gene. Which of the paralogues of SMAD2 is due to the most recent duplication event?

(d) Retrieve an alignment between members of any node using Jalview.

Exercise 2 - Rhodopsins

The photoreceptor cells in the retina of the human eye contain a number of different photoreceptors. The rod cells contain rhodopsin, which is responsible for monochromatic vision in the dark. The cone cells all contain one of three types of opsins, which respond to long-wave (red), middle-wave (green) and short-wave (blue) light, respectively, and are responsible for trichromatic color vision (see for instance http://en.wikipedia.org/wiki/Opsin).

(a) Find the gene encoding the red-sensitive opsin.

(b) Which paralogue has the most sequence similarity with the red-sensitive opsin? (Click on the Target or Query % id column to order the choices from higher percentage to lower).

(c) Have a look at the genomic location of the red-, green- and blue-sensitive opsin genes. (You can do a new search for the blue-sensitive opsins). Does this explain why red-green colour blindness is much more prevalent in males than in females (e.g. in the US population 7% vs 0.4%)?

(d) If you were browsing with the OPN1SW gene, how could you find out it is a “blue-sensitive opsin”?

Exercise 3 – Multi-species Alignments

Find the Ensembl F9 gene for human and go to the **Region in detail** page (in the Location tab).

(a) Turn on the cow and mouse BLASTZ alignment tracks along with the frog (Xenopus tropicalis) Translated BLAT alignment track. BLASTZ is used to compare two species on the nucleotide level. In which species do you see the most aligned sequence?

(b) Turn on the three tracks for the multispecies 34 eutherian mammals alignments. These are found in "configure this page", "Multiple alignments". You can read more about these conservation scores and constrained elements in the "Comparative Genomics" documentation (under the "Documentation" link at the top of the page). How do these tracks differ from the pairwise alignments you looked at in (a)?

ANSWERS COMPARATIVE GENOMICS

Answer 1

Under '**Search Ensembl**' type '**human gene SMAD2**' and click **[Go]**. On the page with search results click on 'Ensembl protein_coding Gene: **ENSG00000175387** (HGNC (curated): SMAD2)'.

(a) Click on **'Paralogues'** in the side menu.

There are six within-species paralogues predicted for human SMAD2. The first one (SMAD3) has the highest Target %id and Query %id. (Not sure what these are? Click on the Help button, and then 'Glossary' in the resulting window.)

Click on the "Alignment" link for a paralogue.

(b) Click on **'Orthologues'** in the side menu.

Yes, there is an orthologue predicted for human SMAD2 in gorilla: ENSGGOG00000004706 (SMAD2).

(c) Click on **'Gene Tree (image)'** in the side menu.
Click on **'View paralogs of current gene'** under the figure.

The gene of interest is in red, and paralogues should be shown in blue.

Click on the nodes (red squares) for the duplication events that have given rise to the various paralogues.

SMAD3 is related by a duplication on the level of the "Euteleostomi (Bony vertebrates)". Click on the common ancestor (red node) to see this.

(d) Click on the duplication node (red square) or speciation node (blue square) of any sub-tree that you are interested in.

In the pop-up menu click on **[Start Jalview].**

To edit the alignment display, you can remove sequences using the option Edit > Delete in the menu bar. Note the other available edit options, e.g. Remove Empty Columns.

Answer 2

(a) Go to the Ensembl homepage (http://www.ensembl.org).
Type **'human red-sensitive opsin gene'** in the **'Search: for'** text box.
Click [Go].
Click on **'*Homo sapiens*'** on the page with search results.
Click on **'Gene'.**
Click on **'Ensembl protein_coding Gene: ENSG00000102076 (HGNC (curated): OPN1LW)**'.

'LW' in the gene symbol OPN1LW stands for 'long-wave'.

(b) Click on '**Comparative Genomics - Paralogues**' in the side menu.

ENSG00000147380 (OPN1MW) and ENSG00000166160 (OPN1MW2), the genes encoding the green-sensitive (middle-wave) opsins, have the highest Target %id and Query %id.

(c) Look at the Location of '**ENSG00000166160**', '**ENSG00000147380**' and '**ENSG00000128617**'. These will be listed in the Paralogue table.

The genes for the red and green-sensitive opsins are located next to each other on the X chromosome, while the gene for the blue-sensitive Opsin (OPN1SW) is located on chromosome 7. As females have two X chromosomes a normal, gene on one chromosome can often make up for a defective one on the other, whereas males cannot make up for a defective gene. Thus, red-green colour blindness is much more prevalent in males than in females. Variations in the genes for red and green-sensitive opsins can cause subtle differences in colour perception, while tandem rearrangements due to unequal crossing-over between these genes cause more serious defects in colour vision.

(d) To find gene (protein) functions, go to the transcript tab, "General identifiers" section. In the UniProt and RefSeq descriptions, blue-light sensitivity is listed.

Answer Exercise 3

Under '**Search Ensembl**' type '**human gene F9**' and click **[Go].**
On the page with search results click on 'Ensembl protein_coding Gene: **ENSG00000101981** (HGNC symbol: F9)'. *(Hint: if you click directly on*

"Region in Detail" from the search results, you don't have to go through the Gene tab.)
Click on the **Location tab.**
Click on '**Configure this page'** in the side menu
Click on '**BLASTz/LASTz alignments**', select '**Cow (*Bos Taurus*)**' and **'Mouse (*Mus musculus*)'**, click on **'Translated BLAT'** alignments, select **'*Xenopus tropicalis*'** and close the menu.

(a) Species that are closer to human in evolution show a larger extent of conservation. The cow and mouse alignments show high 'coverage', meaning that alignments were found to a large percent of the human genome sequence. The *Xenopus* (frog) alignment matches to some exons of F9. This reflects the Translated BLAT approach, where the genome is translated and then aligned. Alignments are focused on protein sequences. Of course, the quality of the alignment reflects the quality of the genome sequence.

(b) The pink bar corresponding to the "34 eutherian mammal" alignment shows the full region aligned across 34 mammals. Click on the track name (34 eutherian mammals) to see the mammals used in this alignment. The 'Conservation score' (34 way GERP score) is calculated from this multispecies alignment. The score shows how well each nucleotide is conserved across the 34 species. A positive score reflects a high conservation. The individual scores can be seen in the plot. Above the plot are the 'constrained elements'. These show sequences in the alignment with high GERP scores. (High GERP scores signify conserved regions.) Many of the conserved elements match up to exons, but there are also constrained elements in introns. These could be important functional sequences.

In summary, the blocks in the 34 way GERP elements track are the most conserved parts of the genome, when comparing 34 mammals. Keep in mind, this alignment includes the low-coverage (low quality) genome sequences.

VII) EXERCISES VARIATIONS AND FUNCTIONAL GENOMICS

Exercise 1

A non-synonymous SNP, R620W (C1858T), in PTPN22 (Tyrosine-protein phosphatase non-receptor type 22) has been identified as a genetic risk factor for a few diseases.

(a) Find the Ensembl page with information for this SNP.
(b) What is the minor allele of this SNP in Caucasians (CEU populations)?
(c) Is this minor allele (in (b)) associated with any diseases?

Exercise 2

Find the **Genetic Variation - Comparison image** page for human PTPN22 (use transcript PTPN22-001).

(a) Do both individuals (Venter and Watson) have sequence known at the position of the R620W (C1858T) SNP? Look at "Resequence coverage" to answer this question.

(b) Does either individual have the minor allele?

Exercise 3

Use BioMart to generate an Excel spreadsheet that contains the following information on all SNPs in the transcripts of the human PTPN22 gene: reference ID, alleles (both nucleotides and amino acids), location (both in transcript and in protein) and consequence to the transcript.

Note: you can start with the Ensembl gene database, filter for the PTPN22 gene and then select your attributes from the 'Variations' attributes page.

Exercise 4 Gene regulation: STX7

(a) Find the Location tab, "Region in Detail" page for the STX7 gene. Are there regulatory features in this gene region? If so, where in the gene do they appear?

(b) Click on a grey box at the 5' terminal end of the STX7 transcripts (remember, this is one of the rightmost boxes as the STX7 gene is on the reverse strand). A pop-up box should appear, listing an ID such as "ENSR00000131372" for this feature. Click on the ID to open the Regulation tab.

In what cell lines is the core evidence found?

(c) Click on the gold exon that is shown just under the blue bar. Follow the link to ENSG00000079950. Click on the "Regulation" link at the left of the gene tab.

ANSWERS VARIATIONS AND FUNCTIONAL GENOMICS

Answer 1

Go to the Ensembl homepage.
Under '**Search Ensembl**' type '**human gene PTPN22**' and click **[Go]**.
On the page with search results click on 'Ensembl protein_coding Gene: **ENSG00000134242** (HGNC symbol: PTPN22)'.

(a) Click on '**Variation Table**' in the side menu.
Select "**Show**" in front of "Non-synonymous coding" variations, in the table.

Two of the four PTPN22 transcripts contain a SNP with amino acid (aa) change W/R and aa co-ordinate 620. The SNP rs2476601 is the one we are looking for.

(b) Click on '**rs2476601**'.
Click on '**Population genetics**''.

In Caucasians (CSHL-HAPMAP:HapMap-CEU population) the minor allele is A.

(c) Click '**Phenotype Data**' at the left of the Variation page. The allele A is associated with Type 1 Diabetes, however, T and G are also risk alleles. The A allele is associated with Vitiligo.

Answer Exercise 2

(a) Click on the '**Gene: PTPN22**' tab.
Click on '**ENST00000359785**'.
Click on '**Comparison image**' in the side menu.
There is re-sequencing coverage for both Venter and Watson (grey bars).

(b) Click on '**Configure this page**' in the side menu.
Under '**Select Variation Type**', deselect all options except '**Non-synonymous**'.

The reference sequence shows the minor allele (A) at this position. Click on the green box corresponding to this SNP to view the allele.

Neither Venter nor Watson is homozygous for the minor allele (A) of rs2476601, which predisposes one for rheumatoid arthritis.

Watson is heterozygous at the position for rs2476601.

Answer Exercise 3

Go to the Ensembl homepage
Click the BioMart link on the toolbar.

Choose the 'Ensembl Genes 60' database. *(Note, you can also do this query using the 'Ensembl Variation 60' database.)*
Choose the 'Homo sapiens genes (GRCh37)' dataset.

Click on 'Filters' in the left panel.
Expand the 'GENE' section by clicking on the + box.
Select 'ID list limit – HGNC symbol' and enter 'PTPN22' in the text box.

Click on 'Attributes' in the left panel.
Select the 'Variations' attributes page.

Expand the 'GENE ASSOCIATED VARIATIONS' section by clicking on the + box.
Select, in addition to the attribute 'Reference ID' that is already default selected, 'Allele', 'Transcript location (bp)', 'Protein location (aa)', and 'Protein Allele'.

Click the [Results] button on the toolbar.
View all rows, or select 'Export all results to file – XLS' (unique results only) and click [Go].

Answer Exercise 4

(a) Search for "**human gene STX7**" from the home page. Click on "Region in detail" from the search results.

Regulatory features from the Ensembl "regulatory build" are based on indicators of open chromatin such as CTCF binding sites, DNase I hypersensitive sites, and transcription factor binging sites. Find them in the "Reg. Feats" track. Click on the "Reg. Feats" track name to jump to an article explaining the underlying data.

Shaded grey boxes indicate regulatory features. There are quite a few of them mapping to the STX7 transcripts, including the 5' end.

(b) At the top of the Regulation tab, a list of cell lines is shown. Core evidence is available for 10 cell lines, including CD4. "Promoter associated" indicates there are promoter-like histone modification patterns for that cell type. The evidence described in the top list are shown graphically in the first panel under "Details by cell line". These are shown as green blocks.

Below the multi-cell graphic, evidence for each cell line is shown in separate panels.

(c) The regulation page shows elements associated with gene regulation from multiple sources. Multiple "regulatory features" are show as shaded boxes in the diagram, and are listed in the table below. In addition, miRNA targets from the miRanda project are indicated, and CisRED motifs are shown.

VIII) EXERCISES GENEBUILD

Exercise 1

(a) From where is the human genome assembly?
(b) How long did it take for Ensembl to perform the last gene build?
(c) How many protein coding genes are there in human? Can you get this same number using BioMart?

Exercise 2

Find the Ensembl GALP (Galanin-like peptide precursor) gene for human.

(a) From what source did Ensembl get the name for this gene? And from where did it get the description 'Galanin-like peptide Precursor'?
(b) On how many pieces of evidence has the transcript of this gene been built?
(c) Why do some pieces of evidence not support the first exon of the transcript?

Exercise 3

Find the Ensembl Epc1 (enhancer of polycomb homolog 1) gene for mouse.

(a) How many transcripts has Ensembl annotated for this gene?
(b) How many transcripts have the manual annotators (Havana) annotated for this gene?
(c) How many transcripts agree between Ensembl and Havana annotation?
(d) What is the reason that Ensembl hasn't found one of the Havana transcripts?

Exercise 4

An example of what can go wrong ….
Go to the following page in Ensembl release 46 (of August 2007):

http://aug2007.archive.ensembl.org/Homo_sapiens/geneview?gene=ENSG00000198561

(a) What is wrong with this gene? What could be the reason for this?
(b) Has this problem been fixed in Ensembl release 60?

ANSWERS GENEBUILD

Answer 1

Go to http://www.ensembl.org.
Click on the human picture or the word 'Human' next to it.

(a) GRC (the Genome Reference Consortium) hosts the assembly determined from the IHGP (International Human Genome Project).

(b) Click on '**Assembly and Genebuild**' in the side menu.
Three months (from March 2009 until May 2009).

(c) Look further down the table. **21,077 known** and **521 novel** protein coding genes. Get the same number in BioMart by using the Filter: GENE panel:

Gene Type: Protein coding
Status(gene): Known
(click count).
Change status to Novel
(click count).

Answer 2

Go to the Ensembl homepage.
Under '**Search Ensembl**' type '**human gene GALP**' and click [Go].
On the page with search results click on 'Ensembl protein_coding Gene: **ENSG00000197487** (HGNC Symbol: GALP)'.

(a) From the HUGO Gene Nomenclature Committee (HGNC). Click on the hyperlinked 'GALP' after 'Name' to see the HUGO gene card.

(b) Click on the '**Transcript: GALP-201**' tab.
Click on '**Supporting evidence**' in the side menu.

Two main pieces of evidence, **NM_033106.2**, which is a 'known mRNA' in NCBI's RefSeq set, and **CCDS12940.1**, which is a coding sequence from the CCDS set. To view these records, click on the diagram representing the sequences and follow the link to the ID. Seven other mRNA and protein sequences are drawn below- these contributed or also aligned well to the Ensembl transcript.

(c) The three pieces of protein evidence (**NP_001139018, Q9UBC7_1** and **Q9UBC7_2**) as well as the CCDS evidence (**CCDS12940**) don't support the first exon of the GALP transcript, because this exon is a completely untranslated region (it is represented by an unfilled box). Thus, protein sequences and coding sequences alone cannot provide any information for this exon.

Answer 3

Go to the Ensembl homepage.
Under '**Search'** type '**mouse gene Epc1**' and click **[Go].**
On the page with search results click on 'Ensembl protein_coding Gene: **ENSMUSG00000024240** (MGI Symbol: Epc1)'.

(a) Four.

(b) Click on '**Configure this page**' in the side menu.
Click on 'Other genes', select 'Vega Havana gene – Expanded with labels' and click [SAVE and close].

There are **four** VEGA-Havana transcripts.

(c) Three (these are the 'gold' transcripts.)

(d) Click on the Epc-004 transcript in the figure.
Click on 'ENSMUST00000124926' in the pop-up menu.
Click on 'Supporting evidence' in the side menu.

In this case the reason is that the transcript ENSMUST00000124926 is built on EST evidence and short cDNA fragments. As Ensembl doesn't build on just EST evidence, it hasn't annotated this transcript in the automatic pipeline, but displays it as a Havana transcript.

Answer 4

Go to
http://aug2007.archive.ensembl.org/Homo_sapiens/geneview?gene=ENSG00000198561

(a) This is the way Ensembl used to look! The gene has two HGNC symbols associated with it, CTNND1 and TXNDC14. The culprit is one long transcript O60716-27(ENST00000360682) that connects two transcript clusters.

(b) Go to the current Ensembl homepage at www.ensembl.org

Under 'Search Ensembl' type 'human gene CTNND1' and click [Go].
On the page with search results click on 'Ensembl protein_coding Gene: ENSG00000198561 (HGNC Symbol: CTNND1)'.
The gene only has one HGNC name, a good indicator of proper annotation.

Click on the 'Location' tab.
Zoom out two steps, so both the CTNDD1 transcripts and the TMX2 (formerly TXNDC14) transcripts are shown.

In Ensembl release 60, CTNND1 and TMX2 are annotated as separate genes.

IX) EXERCISES AND ANSWERS - USER UPLOAD

Exercise 1 – Attaching a file

Have a look at the following file:

http://www.ebi.ac.uk/~bert/n-scan_genes.txt

It contains annotations for three transcripts of the human HFE gene (ENSG00000010704) generated by the N-SCAN gene structure prediction software, as shown on the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgTrackUi?hgsid=148199581&c=chr22&g=nscanGene).

The file is in GFF (General Feature Format) format:

http://genome.ucsc.edu/FAQ/FAQformat

Attach the file to Ensembl and have a look at the result.

Answer

- Go to the Ensembl homepage (http://www.ensembl.org).
- Select 'Search: Human' and type 'hfe gene' in the 'for' text box.
- Click [Go].
- Click on 'Gene' on the page with search results.
- Click on 'Homo sapiens'.
- Click on '[Region in detail]' behind 'Ensembl protein_coding Gene: ENSG00000010704 (HGNC Symbol: HFE)'.
- Click [Manage your data] in the side menu.
- Click on 'Attach URL Data'.
- Enter the URL of the file in the 'File URL' text box.
- Enter 'N-SCAN genes' in the 'Name for this track' text box.
- Click [Next>].
- Click [✓].

A new track named 'N-SCAN genes' should now have been added to the Region in detail page.

To display the names of the transcripts:

- Click [Configure this page] in the side menu.
- Select 'N-SCAN genes - Labels'.
- Click [✓].

Note that, at the moment, the CDS information in the GFF file is not taken into account in Ensembl and thus no distinction between the UTR and CDS of the transcripts can be seen.

Exercise 2 – Uploading data

Have a look at the following file:

http://www.ebi.ac.uk/~bert/medip-chip_cd4.txt

It contains methylation values in the genomic region of the human ICAM3 gene (ENSG00000076662) in CD4 cells (Rakyan *et al.* Genome Res. 2008 Sep;18(9):1518-29).

The file is in BED (Browser Extensible Data) format:

http://genome.ucsc.edu/FAQ/FAQformat

Note that only the chrom, chromStart, chromEnd and score have been used in this case. To display the data as a histogram the useScore attribute in the track line is set to 3.

Upload the data to Ensembl and have a look at the result.

MeDIP data for CD4 cells is also available as a DAS track in Region in Detail. Can you find it?

Answer

- Go to the Ensembl homepage (http://www.ensembl.org).
- Select 'Search: Human' and type 'icam3 gene' in the 'for' text box.
- Click [Go].
- Click on 'Gene' on the page with search results.
- Click on 'Homo sapiens'.
- Click on '[Region in detail]' behind 'Ensembl protein_coding Gene: ENSG00000076662 (HGNC Symbol: ICAM3)'.
- Click [Manage your data] in the side menu.
- Click on 'Upload Data'.
- Enter 'MeDIP-chip CD4' in the 'Name for this upload (optional)' text box.
- Copy/paste the data from the medip-chip_cd4.txt file in the 'Paste file' text box.
- Click [Upload].
- Click [✓].

A new track named 'MeDIP-chip CD4' should now have been added to the Region in detail page.

- Click [Configure this page] in the side menu.
- Search the menu for MeDIP
- Turn on the track for MeDIP-CD4
- Click [✓].

A second track should now appear for the MeDIP-chip data.

Exercise 3 – Creating and uploading an annotation file

Create a small text file containing some annotation in either BED or GFF format (http://genome.ucsc.edu/FAQ/FAQformat) and upload it to Ensembl.

Note that BED offers the simplest format, with only three required fields, i.e. chrom, chromStart and chromEnd, so it's probably easiest to start with this.

Answer

- Create a text file with your annotation in for example Notepad or TextEdit and save it on your computer.
- Go to the region you have annotated in Region in detail.
- Click [Manage your data] in the side menu.
- Click on 'Upload Data'.
- Enter the name for your track in the 'Name for this upload (optional)' text box.
- Click [Browse…] behind 'Upload file:'.
- Select the text file you just created.
- Click [Upload].
- Click [✓].

Your data should now be shown as a new track on Region in detail.

Exercise 5 – Removing custom annotation

Remove your attached and uploaded annotations.

Answer

- Click [Manage your data] in the side menu.
- Click for each dataset on 'Delete'.
- Click [✓].

Your annotations should be removed now.

X) TYING IT TOGETHER- THE HUMAN LDLR GENE

You are working with a transcript of the human **LDLR** gene that has 18 exons. Start at *www.ensembl.org*.

1) For what is **LDLR** an acronym (what is the expanded name)?

2) Go to the transcript tab for the 18-exon transcript.

3) Look under **External References** at the left. Find the **General identifiers** section for the transcript.

   - What Swiss-Prot record matches to the Ensembl protein?
     What NCBI RefSeq protein matches to the Ensembl protein? (This will be an ID starting with NP).

   - Does MIM (Online Mendelian Inheritance in Man) show this protein to be related to any diseases?

4) Click **Gene ontology**. What functions are listed for LDLR?

5) Click on **cDNA** at the left. Where does the 5' UTR end (which base pair number, in the transcript sequence?) Are there variations that change the protein sequence?

6) Click on **population comparison** at the left of the transcript tab.

   - For the non-synonymous SNP in Venter's genome, what is the allele in the reference sequence, and what are the alleles in Venter's sequence?

7) Jump to the variation by clicking on the ID (rs11669576) Click on **population genetics** at the left.

   - What genotypes are found in the Yoruba population (CSHL-HAPMAP-YRI)? Do these differ from other populations studied?

8) Click **Gene/Transcript** at the left. This lists Ensembl genes and transcripts affected by the variation.

9) Click on the gene identifier (**ENSG00000130164**).

10) The **Variation Image** should be shown

- To clarify the image, turn off the variation source "HGMD-PUBLIC" in *configure this page*.

- Non-synonymous and synonymous SNPs are shown in yellow and green, respectively. The possible amino acids at each position are shown.

11) Click **Genomic alignments** at the left. Multispecies alignments can be seen in this view.

- Select "6 primates EPO" in the Alignment menu at the top. Click "Go".

- Click configure this page. Change Conservation regions from "none" to "all conserved regions" (if they are not already turned on).

- Click the "6 primates EPO" link at the left of the menu.

- Deselect "Ancestral sequence". Click [✓]

- Blue shading shows identical nucleotides. Can you see the insertion in the Callithrix jacchus (Marmoset)?

- Turn on variations using "configure this page" and the "Variations: yes and show links" option.

- Click [✓] to close.

- Can you find rs11669576 in the sequence (hint: search with your browser and look for the link at the right, and yellow variation)

XI) Quick Guide to Databases and Projects

Here is a list of databases and projects you will come across in these exercises. Google any one of these to learn more. Projects include many species, unless otherwise noted.

SEQUENCES

EMBL-Bank, NCBI GenBank, DDBJ – Contain nucleic acid sequences deposited by submitters such as wet-lab biologists and gene sequencing projects. These three databases are synchronised with each other every day, so the same sequences should be found in each.

CCDS – coding sequences that are agreed upon by Ensembl, VEGA-Havana, UCSC, and NCBI. *(Human and mouse).*

NCBI Entrez Gene – NCBI's gene collection

NCBI RefSeq – NCBI's collection of 'reference sequences', includes genomic DNA, transcripts and proteins.

UniProtKB – the "Protein knowledgebase", a comprehensive set of protein sequences. Divided into two parts: Swiss-Prot and TrEMBL

UniProt Swiss-Prot – the manually annotated, reviewed protein sequences in the UniProtKB. High quality.

UniProt TrEMBL – the automatically annotated, unreviewed set of proteins (EMBL-Bank translated). Varying quality.

VEGA – Vertebrate Genome Annotation, a selection of manually-curated genes, transcripts, and proteins. *(Human, Mouse, Zebrafish, Gorilla, Wallaby, Pig, and Dog).*

VEGA-HAVANA – The main contributor to the VEGA project, located at the Wellcome Trust Sanger Institute, Hinxton, UK.

GENE NAMES

HGNC – HUGO Gene Nomenclature Committee, a project assigning a unique and meaningful name and symbol to every human gene. *(Human).*

ZFIN – The Zebrafish Model Organism Database. Gene names are only one part of this project. *(Z-fish).*

PROTEIN SIGNATURES

InterPro – A collection of domains, motifs, and other protein signatures. Protein signature records are extensive, and combine information from individual projects such as UniProt, along with other databases such as SMART, PFAM and PROSITE (explained below).

PFAM – A collection of protein families

PROSITE – A collection of protein domains, families, and functional sites.

SMART – A collection of evolutionarily conserved protein domains.

OTHER PROJECTS

NCBI dbSNP – A collection of sequence polymorphisms; mainly single nucleotide polymorphisms, along with insertion-deletions.

NCBI OMIM – Online Mendelian Inheritance in Man – a resource showing phenotypes and diseases related to genes *(human).*