

# Access to genes and genomes with Ensembl



**Course Manual**

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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.

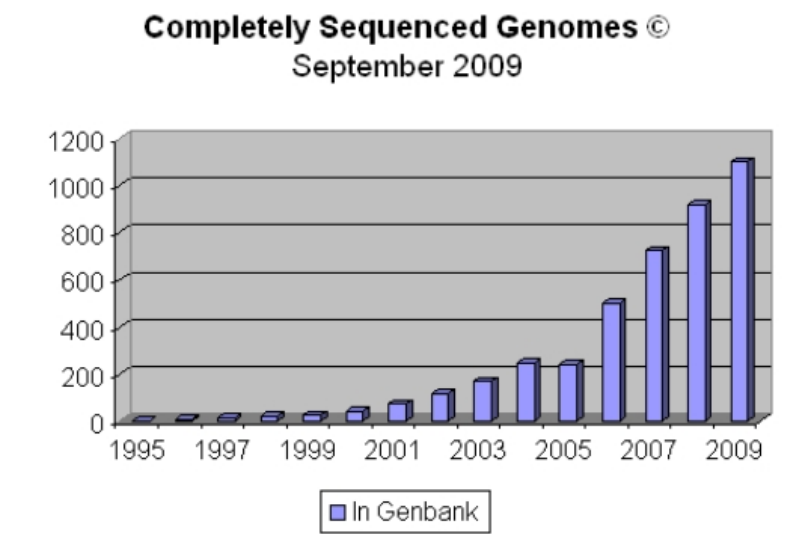


Figure 1. Completely sequenced genomes as of January, 2009 (figure taken from <http://www.genomesonline.org>).

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 2010**

Nucleic Acids Res. Jan 2010 38:D557-D562 (*database issue*)

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

Fernández Suárez X. M. and Schuster M.

#### **Using the Ensembl Genome Server to Browse Genomic Sequence Data.**

*Current Protocols in Bioinformatics*, UNIT 1.15, January 2007.

Hubbard, T.J.P. *et al.*

#### **Ensembl 2007**

Nucleic Acids Res. 2007 (*Database Issue*)

Birney, E. *et al.*<sup>1</sup>

#### **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)

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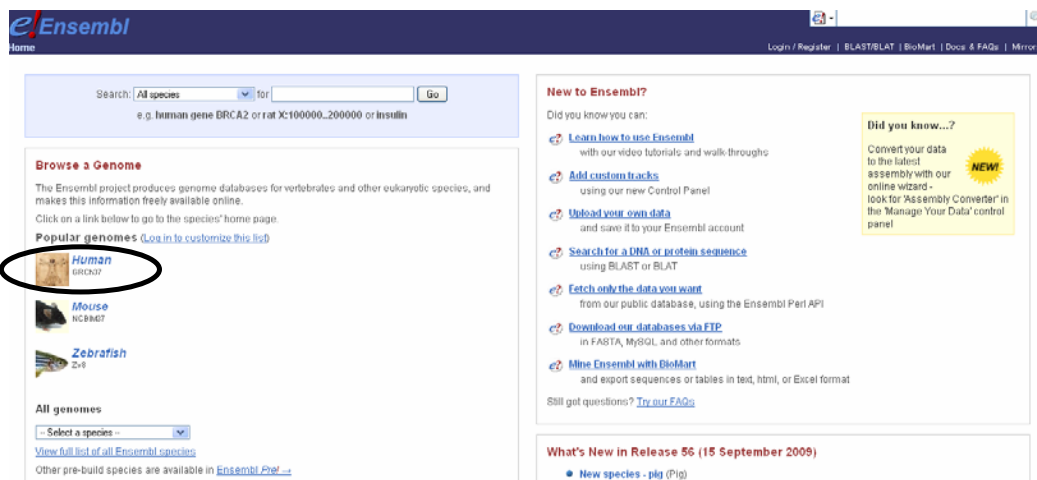
<sup>1</sup> This paper was part of the May 2004 issue of *Genome Research* which included an Ensembl special covering detailed aspects of the Ensembl web site, the underlying scalable database system for storing genome sequence and annotation information, as well as the automated genome analysis and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

**Search Ensembl Human**

Search for:

e.g. gene BRCA2 or AL032821.2.1.143563 or muscular dystrophy

**Description** Assembly and Genebuild >

**Assembly**

This site provides a data set based on the February 2009 *Homo sapiens* high coverage assembly from the [Genome Reference Consortium](#). The data set consist on gene models built from the pairwise alignments of the human proteome as well as from alignments of human cDNAs using the cDNA2genome model of exonerate

This release of the assembly has the following properties:

- 27478 contigs.
- contig length total 3.2 Gb.
- chromosome length total 3.1 Gb.

**Annotation**

Since release 55 (July 2009) the gene annotation presented here has been a combined Ensembl-Havana geneset, which incorporates protein-coding and non-coding transcripts annotated by the Havana team with the Ensembl automatic gene build. The major genome browsers have come together to produce a common set of identifiers where CDS annotations of transcripts can be agreed. This annotation was produced based on the NCBI36 assembly. The CCDS identifiers have been mapped onto the new annotations based on the latest GRCh37 assembly and these identifiers are also shown.

- More information about the [CCDS project](#)

The [ENCODE](#) (ENCyclopedia Of DNA Elements) project aims to find functional elements in the human genome.

- More information about the [ENCODE resources](#) at Ensembl.

**Vega** Additional manual annotation of this genome can be found in [Vega](#)

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

**Search Ensembl** Results Summary Result in Detail >

**By Species**

Species	Count
Total	282
Homo sapiens	282
Gene (282)	

**By Feature type**

Feature type	Count
Total	282
Gene	282

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

Your query matched 282 entries in the search database. Viewing hits 1-10

1 2 3 4 ... 26 27 28 29

**Ensembl protein\_coding Gene: ENSG00000163914 (HGNC (automatic): RHO) [Region in detail]**

Ensembl protein\_coding gene ENSG00000163914 has 1 transcript: ENST00000296271, associated with

ENSE00001152199, ENSE00001152205, ENSE00001152211

**Rho**dopsin (Opsin-2) [Source:UniProtKB/Swiss-Prot;Acc:P08100]

The gene has the following external identifiers mapped to it:

CCDS: CCDS3063, CCDS3063.1

EMBL: BC112106, U16824, U49742, S81166, BX537381, BC112107

EntrezGene: 6010, RP4, **RHO**, MGC138311, CSNBAD1, MGC138300

GO: GO:0016056, GO:0009583, GO:0009416, GO:0007601, GO:0004536, GO:0004583, GO:0007602, GO:0009881, GO:0007186, GO:0009586, GO:0007165, GO:0006468, GO:0018298, GO:0001750, GO:0005622, GO:0005623, GO:0005624, GO:0005625, GO:0005626, GO:0005627, GO:0005628, GO:0005629, GO:0005630, GO:0005631, GO:0005632, GO:0005633, GO:0005634, GO:0005635, GO:0005636, GO:0005637, GO:0005638, GO:0005639, GO:0005640, GO:0005641, GO:0005642, GO:0005643, GO:0005644, GO:0005645, GO:0005646, GO:0005647, GO:0005648, GO:0005649, GO:0005650, GO:0005651, GO:0005652, GO:0005653, GO:0005654, GO:0005655, GO:0005656, GO:0005657, GO:0005658, GO:0005659, GO:0005660, GO:0005661, GO:0005662, GO:0005663, GO:0005664, GO:0005665, GO:0005666, GO:0005667, GO:0005668, GO:0005669, GO:0005670, GO:0005671, GO:0005672, GO:0005673, GO:0005674, GO:0005675, GO:0005676, GO:0005677, GO:0005678, GO:0005679, GO:0005680, GO:0005681, GO:0005682, GO:0005683, GO:0005684, GO:0005685, GO:0005686, GO:0005687, GO:0005688, GO:0005689, GO:0005690, 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Home > Human (GRCh37)

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 [BioMart](#) | 
 [Docs & FAQs](#)

Gene: **RHO**

Gene-based displays

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- External Data
  - Personal annotation
  - History
  - Gene history

Configure this page

Manage your data

Export data

Bookmark this page

Gene: **RHO** (ENS00000163914)

rhodopsin [Source:HGNC Symbol;Acc:10012] Source:

Location [Chromosome 3: 129,247,483-129,254,012](#) forward strand

Transcripts ☒ There are 2 transcripts in this gene

Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CCDS
RHO-001	ENST0000026521	2592	ENSP00000426271	348	Protein coding	CCDS3063
RHO-002	ENST00000511172	454	ENSP00000426267	152	Protein coding	-

[Splice variants](#)

**Gene summary** [help](#)

**Name** [RHO](#) (HGNC Symbol)

**Synonyms** OPN2, RP4 [To view all Ensembl genes linked to the name [click here](#)]

**CCDS** This gene is a member of the Human CCDS set: [CCDS3063](#)

**Gene type** Known protein coding

**Prediction Method** Gene containing both Ensembl genebuild transcripts and [Havana](#) manual curation, see [article](#).

**Alternative genes** This gene corresponds to the following database identifiers:  
Havana gene: [OTTHUMG00000159542](#) [\[view all locations\]](#)

Ensembl/Havana g... 129 24 Mb 129 25 Mb 26.53 kb 129 25 Mb 129 26 Mb Forward strand

IFT122-001 > protein coding  
IFT122-002 > protein coding  
IFT122-006 > protein coding  
IFT122-005 > nonsense mediated decay  
IFT122-021 > protein coding  
IFT122-004 > protein coding  
IFT122-003 > protein coding  
IFT122-002 > protein coding  
IFT122-004 > protein coding  
IFT122-007 > retained intron  
IFT122-009 > retained intron  
IFT122-003 > protein coding  
IFT122-008 > retained intron

Configs 129 24 Mb 129 25 Mb 26.53 Kb 129 25 Mb 129 26 Mb Reverse strand

RHO-001 > protein coding  
RHO-002 > protein coding

HPOG-001 > protein coding

[Export Image](#)

**Configuring the display**

Tip: use the "Configure this page" link on the left to show additional data in this region.

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.

**Gene-based displays**

- Gene summary
- Splice variants (2)
- Supporting evidence
- Sequence**
- External references (2)
- Regulation
- Comparative Genomics
  - Genomic alignments (51)
  - Gene Tree (image)
    - Gene Tree (text)
    - Gene Tree (alignment)
  - Orthologues (106)
  - Paralogues (6)
  - Protein families (1)
- Genetic Variation
  - Variation Table
  - Variation Image
- External Data
  - Personal annotation
- ID History
  - Gene history

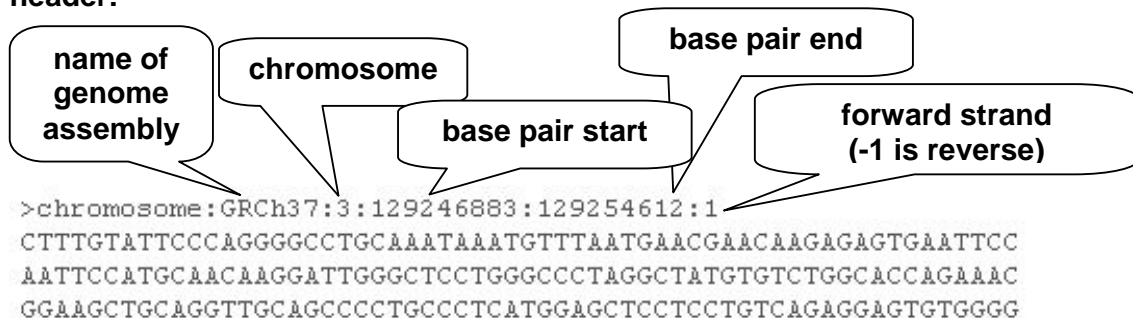
**Click Sequence**

```
>chromosome:GRCh37:3:129246883:129254612:1
CTTTGTATTCCCAGGGGCTGCAAAATAAATGTTTAAATGAACGAACAAGAGAGTGAATTCC
AATTCCATGCAACAAGGATTGGGCTCCTGGGCCCTAGGCTATGTGTCTGGCACCAGAAAC
GGAAGCTGCAGGTTGCAGCCCCCTGCCCTCATGGAGCTCCTCCTGTCAGAGGAGTGTGGGG
ACTGGATGACTCCAGAGGTAACCTGTGGGGGAACGAACAGGTAAGGGGCTGTGTGACGAG
ATGAGAGACTGGGGAATAAACCAGAAAGTCTCTAGCTGTCCAGAGGACATAGCACAGAG
GCCCCATGGTCCCTATTTCAAACCCAGGCCACCAGACTGAGCTGGGACCTTGGGACAGCA
AGTCATGCAGAAATTAGGGGACCTTCTCCTCCCTTTTCTGGATCCTGAGTACCTCTCCT
CCCTGACCTCAGGCTTCTCCTAGTGTACCTTGGCCCTCTTAGAAGCCAATTAGGCC
TCAGTTTCTGCAGCGGGGATTAAATATGATTATGAACACCCCCAATCTCCAGATGCTGAT
TCAGCCAGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTGGGGGGGTCAGAAACCA
GAGTCATCCAGCTGGAGCCCTGAGTGGCTGAGCTCAGGCCCTTCGAGCATTCTTGGGTGG
GAGCAGCCACGGGTGAGCCACAAGGGCCACAGCCATGAATGGCAGAGGGCCCTAACTT
CTACGTGCCCTTCTCCAATGCGACGGGTGTGGTACGACGCCCTTCGAGTACCCACAGTA
CTACCTGGCTGAGCCATGGCAGTTCTCATGCTGGCCGCTACATGTTTCTGCTGATCGT
GCTGGGCTTCCCCATCAACTTCTCACGCTCTACGTACCGTCCAGCACAGAAATGGG
CACGCTCTCAACTACATCTGCTCAACCTAGCCGTGGCTGACCTCTTCATGGTCTTAGG
TGGCTTCACAGCAGCCCTCTACACCTCTCTGATGGATACTTCGTCTTGGGGCCACAGG
ATGCAATTTGGAGGGCTTCTTTGCCACCCCTGGGCGGTATGAGCCGGGTGTGGGTGGGTG
TGCAGGAGCCCCGGGAGCATGGAGGGGTCTGGGAGAGTCCCAGGCTTGGCGGTGGTGGCTG
AGAGGCCCTTCTCCCTTCTCCTGTCTGTCATATGTTATCCAAAGCCCTCATATATTCAGTC
```

Upstream  
sequence

Exon  
sequence

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 [Configure this page](#) link found at the left. Click on [Configure this page now](#).

[Configure this page](#)

The screenshot shows the 'Configure this page' interface for the gene **IL2 (ENSG00000109471)**. The 'Configuration for: "Marked up gene sequence"' section includes the following settings:

- 5' Flanking sequence: 600
- 3' Flanking sequence: 600
- Number of base pairs per row: 60 bps
- Additional exons to display: Core exons
- Orientation of additional exons: Display exons in both orientations
- Show variations: Yes and show links
- Line numbering: Relative to this sequence

Annotations on the screenshot:

- A red circle highlights the **SAVE and close** button in the top right corner.
- An arrow points from the **Show variations** dropdown to a box labeled **Display variations**.
- An arrow points from the **Line numbering** dropdown to a box labeled **Turn on line numbers**.

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

Exons

ENSG00000163914 exons

Ensembl exons in this region

Variations

3' UTR

Intergenic

Splice site SNP

5' UTR

Intronic

Upstream

Downstream

Non-synonymous coding

Link to variation information

>chromosome:GRCh37:3:129246883:129254612:1

1

C T T T G T A T T C C A G G G C C T G C A A A T A A T G T T T A A T G A A C G A A C A A G A G T G A A T T C C

60

61

A A T T C C A T G C A A C A A G G A T T G G G C T C C T G G G C C C T A G G C T A T G T G T C T G G C A C C A G A A A C

120

121

G G A A G C T G C A G G T T G C A G C C C T G C C C T C A T G G A G C T C C T C T G T C A G A G G A G T G T G G G

180

181

A C T G G A T G A C T C C A G A G G T A A C T T G T G G G G A A C A A C A G G T A A G G G C T G T G T A C G A G

240

241

A T G A G A G A C T G G G A A T A A C C A G A A A G T C T A G C T G T C C A G A G A C A T A G C A C A G A G

300

301

G C C C A T G G T C C C T A T T T C A A C C C A G G C C A C C A G A C T G A G C T G G G A C C T T G G G A C A G A C A

360

361

A G T C A T G C A A A G T T A G G G G A C C T T C C T C C C T T T T C C T G G A T C C T G A T A C C T C T C C T

420

421

C C C T G A C C T A G G C T T C C T C T A G T G T C A C C T T G G C C C C T T T A G A A G C C A A T T A G G C C

480

481

T C A G T T T C G A C G C G G G A T T A A T A T G A T T A T G A A C A C C C C A A T C T C C C A G A T G C T G A T

540

541

T C A G C C A G G A G C T T A G G A G G G G A G G T C A C T T T A T A A G G G T C T G G G G G G T C A G A A C C C A

600

601

**G A G T C A T C C A G C T G G A G C C C T G A G T G G C T G A G C T C A G G C C T T C R C A G C A T T C T T G G G T G G**

660

661

**G A G C A G C C R C G G G T C A G C C A C A A G G G C C A C A G C C A T G A A T G G C A C A G A A G G C C C T A A C T T**

720

721

**C T A C G T G C C C T T C C C A T G C G A G G G T G T G G T A C G C A G C C C T T C G A G T A C C C A C A G T A**

780

781

**C T A C C T G G C T G A G C C A T G G C A G T T C C A T G C G G C C G C C T A C A G T T T C T G C T G A T C G T**

840

841

**G C T G G C C T C C C C A T C A A C T T C C T C A G C C T A C G T C A C C G T C A G C A C A G A A G C T G C G**

900

901

**C A C G C C T C T C A A C T A C A C T C T G C T C A A C C T A G C C G T G G C T G A C C T T C T C A T G G T C C T A G C**

960

961

**T G C T T C A C C A G C A C C C T C T A C A C C T C T C T G C A T G G A C T T C G T C T T C R G G C C C A C A G**

1020

1021

**A T G C A A T T T G G A G G C T T C T T T G C C A C C C T G G G C G** G T A T A G C C G G G T G G G T G G G G T G

1080

1081

T G C A G G A G C C C G G A G C A T G A G G G G T C T G G A G A G T C C G G G C C T T G C G G T G G T G C T G

1140

1141

A G A G G C C T T C C C T C C C T G C T G C T G C A A T G T T A T C C A A G C C C T C A T A T A T T C A G T C

1200

644: rs2269736 G/A;

669: rs7984 A/G; 705: CM930

738: CM930648 HGMD MUTATION

813: CM941249 HGMD MUTATION

845: CM930652 HGMD MUTATION

954: CM910329 HGMD MUTATION

963: CM930654 HGMD MUTATION

1022: CM045432 HGMD MUTATION

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 12 eutherian mammals, 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 in the menu.

Homo\_sapiens

GATGCTGATTACGCCAGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTG-----GG---GG-GGTCAGAACCCAGAGTCATCCAGCTC

Pan\_troglodytes

GATGCTGATTACGCCAGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTG-----GG---GG-GGTCAGAACCCAGAGTCATCCAGCTC

Gorilla\_gorilla

GATGCTGATTACGCCAGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTG-----GG---GG-GGTCAGAACCCAGAGTCATCCAGCTC

Pongo\_pygmaeus

GATGCTGATTACGCCAGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTG-----GG---GG-GGTCAGAACCCAGAGTCATCCAGCTC

Macaca\_mulatta

GATGCTGATTACGCCAGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTG-----GG---GG-GGTGAGAGCCAGAGTCATCCAGGCTC

Callithrix\_jacchus

GATGCTGATTACGCCAGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTGTTGGGGGTGGGG---GG-GGTCAGAGCCCGAGTCATCCAGCC

Mus\_musculus

GATGCTGAATCAGCCCTCTGGCTTAGGAGAGAGAAGGTCACTTTATAAGGGTCTG-----GGG---GG-GGTCAGTGCCTGGAGTTGCGCTGTG

Rattus\_norvegicus

GATGCTGAATCAGCCCTCTGGCTTAGGAGAGAGAAGGTCACTTTATAAGGGTCTG-----GGG---GG-GGTCAGTGCCTGGAGTTGCGCTGTG

Bos\_taurus

GGTGCTGATTACGCCGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTG-----GGGGGG---AGTTGGAGCCACAGAGTCGTCCAGCC

Sus\_scrofa

GGTGCTGATTACGCCAGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTG-----GGGGGG---AGTTGGAGCCACAGAGTCGTCCAGCC

Canis\_familiaris

GGTGCTGATTACGCCAGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTG-----GGGGGG---GGTCAGAGCCGAAGTCGTCTCTGCC

Equus\_caballus

GGTGCTGATTACGCCAGGAGCTTAGGAGGGGAGGTCACTTTATAAGGGTCTG-----GGG---GG-GGTCAGAGCCGTGAGTCGTCCAGCC

Homo\_sapiens

GCCTTCGCAGCATTCTTGG-----GTGGGAGCAG-CC-ACGGGTACGCCACAAG-GGCCACAGCCATGAATGGCACAGAAGGCCCTAACTTCTAC

Pan\_troglodytes

GCCTTCGCAGCATTCTTGG-----GTGGGAGCAG-CC-GTGGGTACGCCACAAG-GGCCACAGCCATGAATGGCACAGAAGGCCCTAACTTCTAC

Gorilla\_gorilla

GCCTTCGCAGCATTCTTGG-----GTGGGAGCAG-CC-GCGGGTACGCCACAAG-GGCCACAGCCATGAATGGCACAGAAGGCCCTAACTTCTAC

Pongo\_pygmaeus

GCCTTCGCAGCATTCTTGG-----GTGGGAGCAG-CC-GCGGGTACGCCACAAG-GGCCACAGCCATGAATGGCACAGAAGGCCCTAACTTCTAC

Macaca\_mulatta

GCCTTCGCAGCATTCTTGG-----GTGGGAGCAG-AC-GCGGGGACGCCACAAG-GGCCACAGCCATGAATGGCACAGAAGGCCCTAACTTCTAC

Callithrix\_jacchus

GCCTTTTCAGCATTCTTGG-----GTGGGAGCAG-CC-GTGGGGCAACCCACAAG-GGCCACAGTCATGAATGGCACTGAAGGCCCTAACTTCTAC

Mus\_musculus

CC-----AAGCAGCCTTGGTCTCTGTCTACGAAGAGCC-GTGGGGCAGCCTCGAG-AGCCGACCCATGAAGGCCACAGAGGGCCCCAATTTTAA

Rattus\_norvegicus

CC-----AAGCAGCCTTGGTCTCTGTCTACGAAGAGCC-GTGGGGCAGCCTCAAG-GGCCGACCCATGAAGGCCACAGAGGGCCCCAATTTTAA

Bos\_taurus

GCCTCAGAACATCCCGG-----GTTCGCGCC-CC-GCGGGGACGCCCAAG-GGCCGACCCATGAAGGGGACCGAGGGGCCCAACTTCTAC

Sus\_scrofa

GCCTCAGACGATCCTTGG-----GTTCGCGCC-CC-GCAAGGACGCCACAAGGGGCCGACCCATGAATGGGACCGAGGGGCCCAACTTCTAC

Canis\_familiaris

GCCTCAGACGATCCTTGA-----GACTGAGCC-CCACGGGGGACGCCCAAG-GACCCGACCCATGAACGGGACCGAGGGGCCCAACTTCTAC

Equus\_caballus

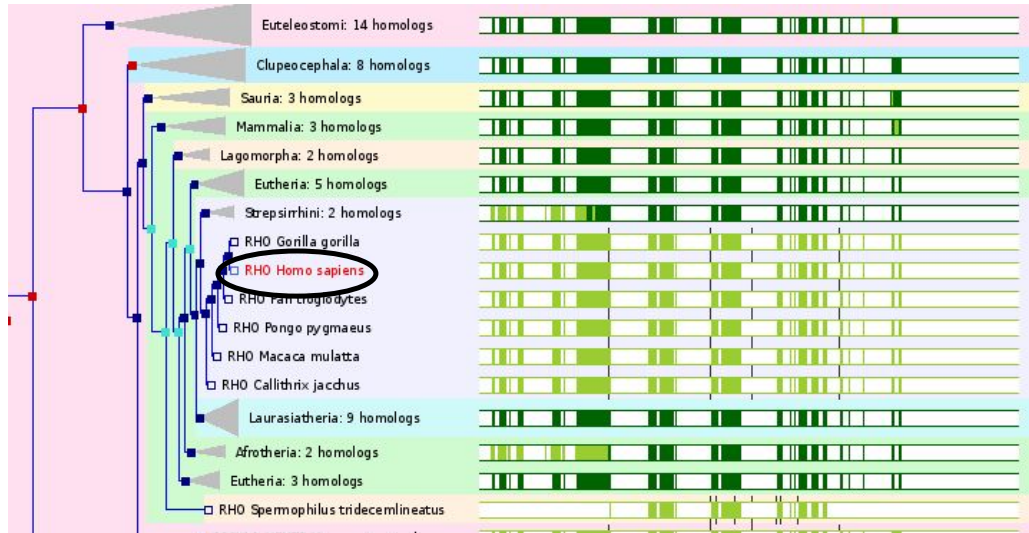
GCCTCAGCAGTATTCTTGG-----GTGGGAGCAG-CCACGGGGGACGCCACAAG-GGCCGACCCATGAACGGGACCGAGGGGCCCAACTTCTAC

11



Exons are highlighted in red, identical 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 to 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 <a href="#">help</a>			Paralogues »		
Species	Type	dN/dS	Ensembl identifier	Location	Target %id	Query %id	External ref.
Alpaca ( <i>Vicugna pacos</i> )	1-to-1	n/a	<a href="#">ENSVPAG00000003334</a> <a href="#">Multi-species view</a> <a href="#">Alignment</a> <a href="#">Gene Tree (image)</a>	<a href="#">GeneScaffold_3012.4867-8438.1</a>	77	77	RHO rhodopsin [Source:HGNC Symbol;Acc:10012]
Anole Lizard ( <i>Anolis carolinensis</i> )	1-to-1	n/a	<a href="#">ENSACAG000000014258</a> <a href="#">Multi-species view</a> <a href="#">Alignment</a> <a href="#">Gene Tree (image)</a>	<a href="#">scaffold_163.632250-640401.1</a>	81	82	OPSD_ANOCA Rhodopsin [Source: UniProtKB/Swiss-Prot; acc: P41591]
Anole Lizard ( <i>Anolis carolinensis</i> )	Possible ortholog	n/a	<a href="#">ENSACAG000000005789</a> <a href="#">Multi-species view</a> <a href="#">Alignment</a> <a href="#">Gene Tree (image)</a>	<a href="#">scaffold_24.1173306-1186146.1</a>	39	33	Novel Ensembl prediction No description
Anole Lizard ( <i>Anolis carolinensis</i> )	Possible ortholog	n/a	<a href="#">ENSACAG000000006891</a> <a href="#">Multi-species view</a> <a href="#">Alignment</a> <a href="#">Gene Tree (image)</a>	<a href="#">scaffold_44.1171109-1177064.1</a>	38	28	Novel Ensembl prediction No description
Anole Lizard ( <i>Anolis carolinensis</i> )	Possible ortholog	n/a	<a href="#">ENSACAG000000008548</a> <a href="#">Multi-species view</a> <a href="#">Alignment</a> <a href="#">Gene Tree (image)</a>	<a href="#">scaffold_1370.18504-26260.1</a>	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.

Then click on the [Variation image](#) (at the left).







Location: 3:129,247,482-129,254,177 Gene: RHO Transcript: RHO-201 Variation: rs28933993

**Transcript: RHO-201 (ENST00000296271)**

rhodopsin [Source:HGNC Symbol;Acc:10012]

**Location** [Chromosome 3: 129,247,482-129,254,177 forward strand.](#)

**Gene** This transcript is a product of gene [ENSG00000163914](#) - There is 1 transcript in this gene: [hide transcripts](#)

Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CCDS
RHO-201	ENST00000296271	2758	ENSP00000296271	348	protein_coding	CCDS3063

« Protein sequence » **General identifiers** [help](#) « Oligo probes »

This KNOWN protein coding entry corresponds to the following database identifiers:

**HGNC Symbol:** [RHO](#)  
rhodopsin [\[view all locations\]](#)

**UCSC Stable ID:** [uc003emt.2](#) [\[view all locations\]](#)

**CCDS:** [CCDS3063.1](#) [\[view all locations\]](#)

**WikiGene:** [RHO](#)  
rhodopsin [\[view all locations\]](#)

**UniProtKB/Swiss-Prot:** [OPSD\\_HUMAN](#) [\[align\]](#)  
Rhodopsin (Opsin-2) [\[view all locations\]](#)

**RefSeq peptide:** [NP\\_000530.1](#) [Target %id: 100; Query %id: 100] [\[align\]](#)  
rhodopsin [\[view all locations\]](#)

**RefSeq DNA:** [NM\\_000539.3](#) [\[align\]](#)  
rhodopsin (RHO), mRNA [\[view all locations\]](#)

**UniProtKB/TrEMBL:** [Q16415\\_HUMAN](#) [Target %id: 6; Query %id: 95] [\[align\]](#)

Other views in the External References menu include microarray probes and gene ontology terms from the GO consortium ([www.geneontology.org](http://www.geneontology.org)).

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

« Comparison image » **Protein summary** [help](#) « Domains & features »

Name	Transcript ID	Length (bp)	Protein ID	Length (aa)	Biotype	CCDS
RHO-201	ENST00000296271	2758	ENSP00000296271	348	Protein coding	CCDS3063

Protein Transmembrane...  
Low complexity...  
Superfamily do...  
Prints domain

PFam domain

PROSITE profiles

PROSITE patterns

Variations

Scale bar

Ensembl protein

Signatures mapped to the sequence

Export image

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 **33 eutherian mammals** (including **"Conservation score"** and **"Constrained elements"**). **Save and 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
AGAGTCATCCAGCTGGAGCCCTGAGTGGCTGAGCTCAGGCCCTTCGCAGCATTCTTGGGTG
GGAGCAGCCACGGGTCAGCCACAAGGGCCACAGCCATGAATGGCACAGAAGGCCCTAACT
TCTACGTGCCCTTCTCCAATGCGACGGGTGTGGTACGCAGCCCTTCGAGTACCCACAGT
ACTACCTGGCTGAGCCATGGCAGTTCTCCATGCTGGCCGCTACATGTTTCTGCTGATCG
TGCTGGGCTTCCCATCAACTTCTCAGCTCTACGTCACGTCACGACACAAGAAGCTGC
GCACGCCCTCTCAACTACATCTGCTCAACCTAGCCGTGGCTGACCTCTTCTGCTCCTAG
GTGGCTTACCAGCACCCTCTACACCTCTCTGCATGGATACTTCTGCTTCGGGCCACAG
GATGCAATTTGGAGGGCTTCTTTGCCACCCTGGGCGGTATGAGCCGGGTGTGGTGGGGT
GTGCAGGAGCCCGGGAGCATGGAGGGGTCTGGGAGAGTCCCGGGCTTGGCGGTGGTGGCT
GAGAGGCCCTTCTCCCTTCTCTGCTGCTCAATGTTATCCAAAGCCCTCATATATTCAGT
CAACAAACACCATTCATGGTGATAGCCGGGCTGCTGTTTGTGCAGGGCTGGCACTGAACA
CTGCCCTTGATCTTATTTGGAGCAATATGCGCTTGTCTAATTTACAGCAAGAAAAGTGA
CTGAGGCTCAAAGAAGTCAAGCGCCCTGCTGGGCGGTACACAGGGACGGGTGCAGAGTT
GAGTTGGAAGCCCGCATCTATCTCGGGCCATGTTTGCAGCACCAGCCTCTGTTTCCCTT
GGAGCAGCTGTGCTGAGTCAGACCCAGGCTGGGCACTGAGGAGAGCTGGGCAAGCCAGA
CCCTCTCTCTGCGGGCCCAAGCTCAGGCTGGGAAGTGGATTTCATTCTCCAGTCAT
TGGGTCTTCCCTGTGCTGGGCAATGGGCTCGGTCCCTCTGGCATCCTTGCCTCCCTC
TCAGCCCTGTCTCAGGTGCCCTCCAGCCCTCCCTGCCGCGTTCAGTCTCCTGGTGT
TGAGAACCGCAAGCAGCCGCTCTGAAGCAGTTCCCTTTTGTCTTGAATAATGCTTGTGA
TTTAAACAGAAAAACAGATGGGCTGCTGCAGGATAACAGATCCCACTTAAACAGAGAGAA
AACTGAGGCAGGGAGAGGGGAAAGAGACTCAATTAGGGATGTGGCCAGGCAGCAACAAGAG
CCTAGGTCTCTGGCTGTGATCCAGGAATATCTCTGCTGAGATGCAGGAGGACGCTAG
```

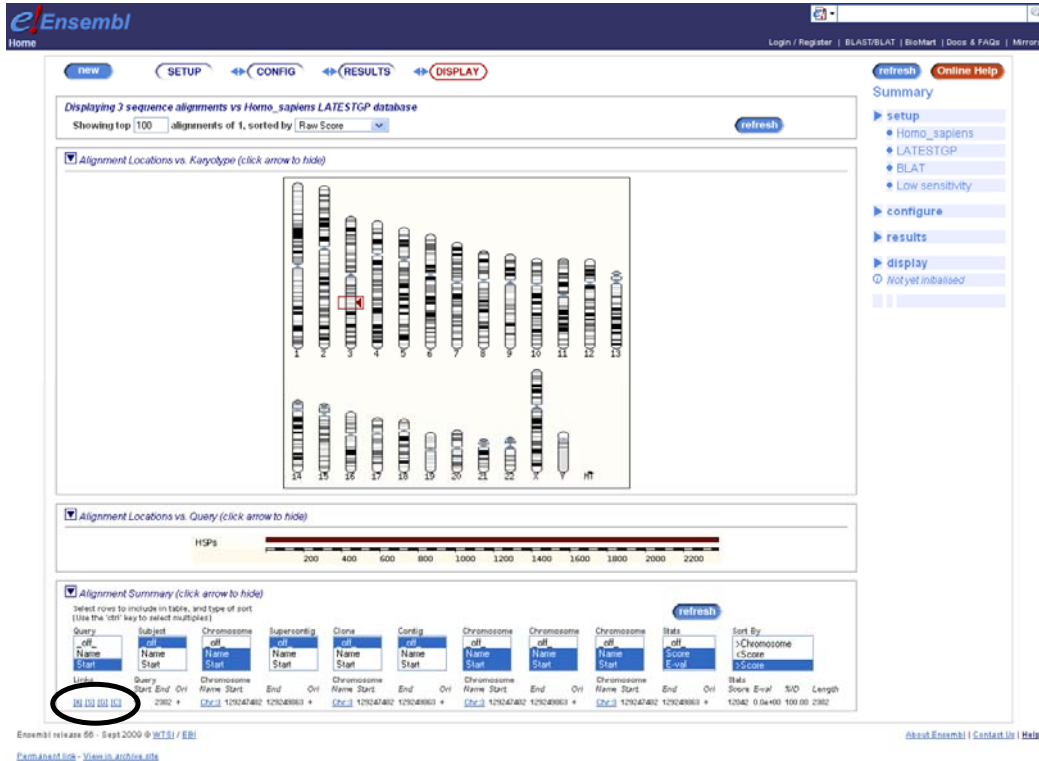
Select the header and a few lines of sequence using Edit/Copy in your browser. Click on the [BLAST/BLAT](#) link in the bar at the top of the page. Paste the sequence into the appropriate box and select **BLAT** as the search algorithm. Finally, click **Run**.

The screenshot shows the Ensembl BLAT/BLAST interface. The top navigation bar includes links for Home, Login / Register, BLAST/BLAT, BioMart, Docs & FAQs, and Mirrors. The main interface has a top navigation bar with buttons for New, SETUP (highlighted), CONFIG, RESULTS, and DISPLAY. On the right, there is a Summary panel with links for setup, configure, results, and display, each with a status indicator (e.g., 'Not yet initialised').

The main content area is divided into several sections:

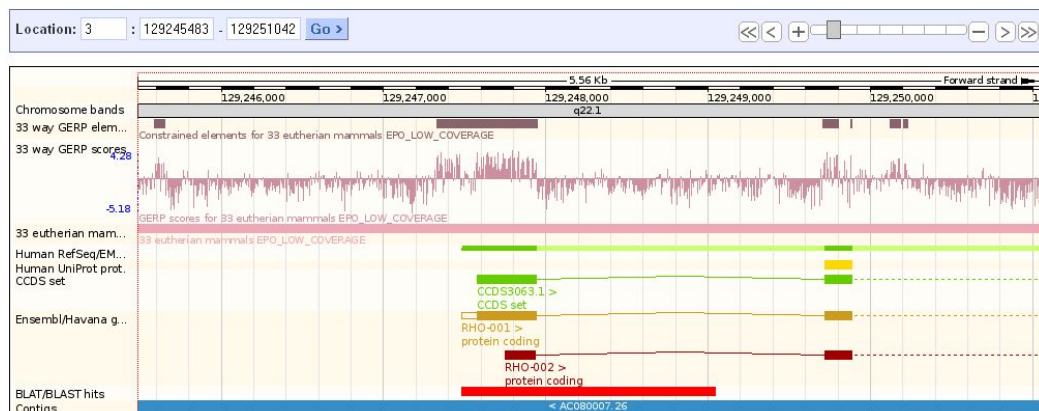
- Important Notice:** A message stating that Ensembl now uses BLAT as its default DNA search engine to make queries faster.
- Enter the Query Sequence:** This section offers three ways to input a query:
  - Paste sequences:** A text area containing the sequence from the previous block, with a 'Paste' button and a 'Clear' button.
  - Upload a file:** A 'Browse...' button to select a file containing one or more FASTA sequences.
  - Enter a sequence ID or accession:** A text input field with a 'Retrieve' button.
  - Enter an existing ticket ID:** A text input field with a 'Retrieve' button.
- Select the databases to search against:**
  - Select species:** A dropdown menu showing 'Gasterocercus aculeatus', 'Gonia, gonia', and 'Mycobacterium'.
  - dna database:** A dropdown menu set to 'LATESTGP'.
  - peptide database:** A dropdown menu set to 'PEP\_ALL'.
- Select the Search Tool:**
  - Buttons for BLASTN, BLAT (highlighted), and TBLASTX.
  - A 'Configure' button and a 'RUN' button.
  - Search sensitivity:** A dropdown menu set to 'Near-exact matches'.
- About Blat/View:** A section providing information about the BLAT/BLAST interface and a link to 'Feedback'.

At the bottom of the page, there is a footer with the text 'Ensembl release 56 - Sept 2009 © EMBL/EBI' and links for 'Feedback', 'View in archive site', 'About Ensembl', 'Contact us', and 'Help'.



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”:**



### III) EXERCISES and ANSWERS

Note: the answers to these exercises correspond to current version (58) of Ensembl. If you use these exercises in the future, after Ensembl is updated, please use the archive site for version 58.

#### 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) Is it possible to monitor expression of ENST00000218099 with the CodeLink microarray?
- (f) Which amino acids in the ENSP00000218099 show peptidase activity?
- (g) How many non-synonymous SNPs have been discovered for the protein encoded by ENST00000218099?
- (h) Is there a mouse ortholog predicted for the human F9 gene?
- (i) On which cytogenetic band and on which contig is the F9 gene located? Is there a BAC clone that contains the complete F9 gene? (To view a definition of contig, click on **Help** and then the **glossary**).

##### 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?

- (b) Do the tilepath clones (i.e. the BAC clones that were sequenced for the human genome assembly) correspond with the contigs?
- (c) Zoom in on the SGCB transcript, including a bit of flanking sequence on both sides.
- (d) Is there a protein from UniProtKB that aligns to the genome to the same location as SGCB?
- (e) 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**' or 'factor IX gene' in the 'for' text box (or alternatively leave the 'Search:' drop-down list like it is and type 'human F9 gene' or 'human factor IX gene' in the 'for' text box). Click [Go]. Click on '**Homo sapiens**' on the page with search results. Click on '**Gene**'. Click on '**Ensembl protein\_coding Gene: ENSG00000101981 (HGNC (curated): 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 (sequence shown in purple).

(c) In the table of transcripts, there are CCDS IDs for F9-001 and F9-201. CCDS is the consensus coding sequence set. These coding sequences have been agreed upon by Ensembl and NCBI.

(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 '**External References - Gene ontology**'.

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<sup>2+</sup> ions, phospholipids, and factor VIIIa (this is the

function description as found in UniProtKB/Swiss-Prot).

**(e)** Click on '**External References - Oligo probes**' in the side menu. The CodeLink microarray contains one probe, GE80895, that maps to ENST00000218099, so it is possible to monitor expression of this transcript using this array.

**(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**. The peptidase activity of the protein resides in the Cterminal half of the protein. Click on one of the "Peptidase\_S1\_S6" bars to see that the domain maps to 226 or 227 to 454 or 459 (the different numbers reflect slightly different lengths of the Peptidase\_S1\_S6 signature in three different databases (SMART, Pfam and PROSITE)).

**(g)** 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.

Note: Many more variations in the F9 protein have been described, but as these haven't been submitted to dbSNP, which is the main source of variation information for Ensembl, they are not shown. They are shown in UniProtKB/Swiss-Prot though, many of them with literature references: <http://www.uniprot.org/uniprot/P00740> (see under 'Sequence annotation (Features) - Natural variations').

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

**(i)** Click on the '**Location: X:138,612,895-138,645,617**' tab. The F9 gene is located on cytogenetic band q27.1 and on contig AL033403.1.

To turn on the clonesets, click on '**Configure this page**' in the side menu. Type '**clone**' in the 'Search display:' text box. Select '**1 Mb clone set – Normal**', '**30k clone set – Normal**', '**32k clone set – Normal**' and '**Tilepath - Normal**'. Tilepath clones are the ones sequenced to make the genomic assembly. Click [**Save and close**].

There are several BAC clones that contain the complete F9 gene, e.g. RP6-88D7, RP11-1144I12 and RP13-520K15.



## 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)** If the tilepath clones are not already shown:

Click on '**Configure this page**' in the side menu.

Type '**tilepath**' in the '**Search display:**' text box.

Select '**Tilepath - Normal**'.

Click **[Save and close]**.

The tilepath clones do correspond to the contigs and it is easy to see from which BAC clone which contig sequence in the assembly is derived, e.g. AC027271.7 is derived from RP11-365H22, AC104784.5 is derived from RP11-61F5 (click on them to see their names) etc. Clones overlap each other, while contigs do not.

**(c)** Click on **SGCB** in the top panel, and follow the link to location. 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.

**(d)** 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**'.

Click **[Save and close]**.

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.

**(e)** 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.

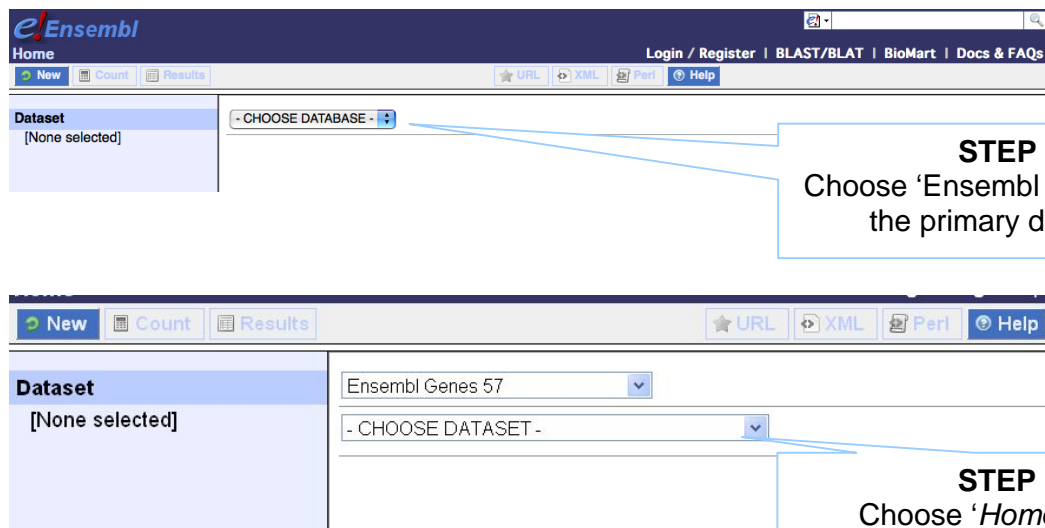
Which other genes with consensus coding sequences assigned by the CCDS project locate to the same band? What are their Ensembl Gene IDs and Entrez Gene IDs?

What are their cDNA sequences?

Follow the worked example below to answer these questions.

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

NOTE: These answers were determined using Ensembl 58. The instructor will have the updated answers for you.



The screenshot shows the Ensembl BioMart interface. The top navigation bar includes links for 'Home', 'Login / Register', 'BLAST/BLAT', 'BioMart', and 'Docs & FAQs'. Below the navigation bar, there are tabs for 'New', 'Count', and 'Results'. The main content area is divided into two sections. The left section, labeled 'Dataset', shows '[None selected]'. The right section contains two dropdown menus. The first dropdown menu is labeled '- CHOOSE DATABASE -' and has a blue arrow pointing to it from a text box. The second dropdown menu is labeled '- CHOOSE DATASET -' and has a blue arrow pointing to it from another text box.

**STEP 2:**  
Choose 'Ensembl Genes 58' as the primary database.

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



**Ensembl**  
Home

Login / Register | BLAST/BLAT | BioMart | Docs & FAQs

New Count Results URL XML Perl Help

Dataset  
Homo sapiens genes (NCBI36)

Filters  
[None selected]

Attributes  
Ensembl Gene ID  
Ensembl Transcript ID

Dataset  
[None Selected]

Please restrict your query using criteria below

REGION:  
GENE:  
EXPRESSION:  
MULTI SPECIES COMPARISONS:  
PROTEIN DOMAINS:  
VARIATIONS:

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

**Ensembl**  
Home

Login / Register | BLAST/BLAT | BioMart | Docs & FAQs

New Count Results URL XML Perl Help

Dataset  
Homo sapiens genes (NCBI36)

Filters  
Chromosome: X  
Band Start: q28  
Band End: q28

Attributes  
Ensembl Gene ID  
Ensembl Transcript ID

Dataset  
[None Selected]

Please restrict your query using criteria below

REGION:  
☒ Chromosome: X  
☐ Base pair: Gene Start (bp): 1, Gene End (bp): 10000000  
☒ Band: Band Start: q28, Band End: q28  
☐ Marker: Marker Start: , Marker End:   
☐ Encode type: manual\_picks  
☐ Encode region: 5:131284314:132284313  
☐ Multiple Chromosomal Regions (Chr:Start:End:Strand): Chromosome Regions:

GENE:  
EXPRESSION:  
MULTI SPECIES COMPARISONS:  
PROTEIN DOMAINS:

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

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

**STEP 7:**  
Expand the 'GENE' panel.

GENE:

☒ Limit to genes ...

with CCDS ID(s)

☒ Only

☐ Excluded

☐ ID list limit

Ensembl Gene ID(s)

Browse...

☐ Transcript count >=

☐ Gene type

IG\_C\_gene  
IG\_D\_gene  
IG\_J\_gene  
IG\_V\_gene  
miRNA

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

New Count Results

Dataset  
Homo sapiens genes (GRCh37)

Filters

Chromosome: X  
Band Start : q28  
Band End : q28  
with CCDS ID(s): Only

Attributes

The 'Count' results show 106 out of  
51,726 total human genes passed the  
filters.

☐ Base pair  
Gene Start (bp)  
Gene End (bp)

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

New Count Results

URL XML Perl

Dataset 105 / 49506 Genes  
Homo sapiens genes (GRCh37)

Filters

Chromosome: X  
Band Start : q28  
Band End : q28  
with CCDS ID(s): Only

Attributes

Ensembl Gene ID  
Ensembl Transcript ID

Dataset  
[None Selected]

Please select columns to be included in the output

☒ Features ☐ Transcript Event  
☐ Structures ☐ Homologs  
☐ Variations ☐ Sequences

GENE:  
EXTERNAL:  
EXPRESSION:  
PROTEIN DOMAINS:

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

**New** **Count** **Results** **URL** **XML** **Perl**

Dataset 105 / 49506 Genes  
Homo sapiens genes (GRCh37)

**Filters**  
Chromosome: X  
Band Start : q28  
Band End : q28  
with CCDS ID(s): Only

**Attributes**  
Ensembl Gene ID  
Ensembl Transcript ID

**Dataset**  
[None Selected]

Please select columns to be included in the output

☒ **Features** ☐ Transcript Event  
☐ Structures ☐ Homologs  
☐ Variations ☐ Sequences

☐ GENE:  
☐ EXTERNAL:  
☐ EXPRESSION:  
☐ PROTEIN DOMAINS:

**STEP 11:**  
Expand the 'GENE' panel.

**New** **Count** **Results** **URL** **XML** **Perl** **Help**

Dataset 105 / 49506 Genes  
Homo sapiens genes (GRCh37)

**Filters**  
Chromosome: X  
Band Start : q28  
Band End : q28  
with CCDS ID(s): Only

**Attributes**  
Ensembl Gene ID  
Ensembl Transcript ID  
Associated Gene Name

**Dataset**  
[None Selected]

Please select columns to be included in the output and hit 'Results' when ready

☒ **Features** ☐ Transcript Event  
☐ Structures ☐ Homologs  
☐ Variations ☐ Sequences

☐ GENE:  
☐ EXTERNAL:  
☐ EXPRESSION:  
☐ PROTEIN DOMAINS:

**Ensembl**  
☒ Ensembl Gene ID  
☒ Ensembl Transcript ID  
☐ Ensembl Protein ID  
☐ Canonical transcript stable ID(s)  
☐ Description  
☐ Chromosome Name  
☐ Gene Start (bp)  
☐ Gene End (bp)  
☐ Strand  
☐ Band  
☐ Transcript Start (bp)  
☐ Transcript End (bp)

☒ Associated Gene Name  
☐ Associated Transcript Name  
☐ Associated Gene DB  
☐ Associated Transcript DB  
☐ Transcript count  
☐ % GC content  
☐ Gene Biotype  
☐ Transcript Biotype  
☐ Source  
☐ Status (gene)  
☐ Status (transcript)

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).

**New** **Count** **Results** **URL** **XML** **Perl** **Help**

Dataset 105 / 49506 Genes  
Homo sapiens genes (GRCh37)

**Filters**  
Chromosome: X  
Band Start : q28  
Band End : q28  
with CCDS ID(s): Only

**Attributes**  
Ensembl Gene ID  
Ensembl Transcript ID  
Associated Gene Name

**Dataset**  
[None Selected]

Please select columns to be included in the output and hit 'Results' when ready

☐ **Features** ☐ Transcript Event  
☐ Structures ☐ Homologs  
☐ Variations ☐ Sequences

☐ GENE:  
☐ EXTERNAL:  
☐ EXPRESSION:  
☐ PROTEIN DOMAINS:

**Ensembl**  
☒ Ensembl Gene ID  
☒ Ensembl Transcript ID  
☐ Ensembl Protein ID  
☐ Canonical transcript stable ID(s)  
☐ Description  
☐ Chromosome Name  
☐ Gene Start (bp)  
☐ Gene End (bp)  
☐ Strand  
☐ Band  
☐ Transcript Start (bp)  
☐ Transcript End (bp)

☒ Associated Gene Name  
☐ Associated Transcript Name  
☐ Associated Gene DB  
☐ Associated Transcript DB  
☐ Transcript count  
☐ % GC content  
☐ Gene Biotype  
☐ Transcript Biotype  
☐ Source  
☐ Status (gene)  
☐ Status (transcript)

**STEP 13:**  
Expand the 'EXTERNAL' panel to select External References.

**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) (OTTT)

☐ Ensembl transcript (where OTTT shares CDS with OTTT)

☐ HAVANA transcript (where ENST shares CDS with OTTT)

☐ 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/IGH-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

☐ Human Protein Atlas Antibody 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'. These are diseases from NCBI's OMIM project.

**Ensembl**  
Home

Dataset 105 / 49506 Genes

Homo sapiens genes (GRCh37)

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

[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
ENSG00000166049	ENST00000370357	PASD1	139135		
ENSG000000010404	ENST00000370441	IDS	3423		
ENSG000000010404	ENST00000340855	IDS	3423	309900	MUCOPOLYSACCHARIDOSIS TYPE II
ENSG00000124334	ENST00000244174	IL9R	3581		
ENSG00000124333	ENST00000288448	VAMP7	6845		
ENSG00000168939	ENST00000369437	RYR3	10251		
ENSG00000185973	ENST00000334398		55217		
ENSG00000185978	ENST00000369444		83740		
ENSG00000185978	ENST00000369444		474381		
ENSG00000185990	ENST00000369445		8263		

**STEP 15:**

Click 'RESULTS' at the top to preview the output.

**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 to any address.

Ensembl Gene ID	Ensembl Transcript ID	Associated Gene Name	Entrez Gene ID	MIM Morbid Accession	MIM Morbid Description
<a href="#">ENSG00000166049</a>	<a href="#">ENST00000370357</a>	<a href="#">PASD1</a>	<a href="#">139135</a>		
<a href="#">ENSG0000010404</a>	<a href="#">ENST00000370441</a>	<a href="#">IDS</a>	<a href="#">3423</a>		
<a href="#">ENSG0000010404</a>	<a href="#">ENST00000340855</a>	<a href="#">IDS</a>	<a href="#">3423</a>	<a href="#">309900</a>	MUCOPOLYSACCHARIDOSIS TYPE II
<a href="#">ENSG00000124334</a>	<a href="#">ENST00000244174</a>	<a href="#">IL9R</a>	<a href="#">3581</a>		
<a href="#">ENSG00000124333</a>	<a href="#">ENST00000286448</a>	<a href="#">VAMP7</a>	<a href="#">6845</a>		
<a href="#">ENSG00000168939</a>	<a href="#">ENST00000369437</a>	<a href="#">SPRY3</a>	<a href="#">10251</a>		
<a href="#">ENSG00000185973</a>	<a href="#">ENST00000334398</a>	<a href="#">TMLHE</a>	<a href="#">55217</a>		
<a href="#">ENSG00000185978</a>	<a href="#">ENST00000369444</a>	<a href="#">H2AFB3</a>	<a href="#">83740</a>		
<a href="#">ENSG00000185978</a>	<a href="#">ENST00000369444</a>	<a href="#">H2AFB3</a>	<a href="#">474381</a>		
<a href="#">ENSG00000185990</a>	<a href="#">ENST00000369445</a>	<a href="#">F8A3</a>	<a href="#">8263</a>		
<a href="#">ENSG00000185990</a>	<a href="#">ENST00000369445</a>	<a href="#">F8A3</a>	<a href="#">474383</a>		
<a href="#">ENSG00000185990</a>	<a href="#">ENST00000369445</a>	<a href="#">F8A3</a>	<a href="#">474384</a>		
<a href="#">ENSG00000198444</a>	<a href="#">ENST00000369505</a>	<a href="#">F8A2</a>	<a href="#">8263</a>		
<a href="#">ENSG00000198444</a>	<a href="#">ENST00000369505</a>	<a href="#">F8A2</a>	<a href="#">474383</a>		
<a href="#">ENSG00000198444</a>	<a href="#">ENST00000369505</a>	<a href="#">F8A2</a>	<a href="#">474384</a>		
<a href="#">ENSG00000198307</a>	<a href="#">ENST00000354514</a>	<a href="#">H2AFB2</a>	<a href="#">83740</a>		
<a href="#">ENSG00000198307</a>	<a href="#">ENST00000354514</a>	<a href="#">H2AFB2</a>	<a href="#">474381</a>		
<a href="#">ENSG00000155962</a>	<a href="#">ENST00000369449</a>	<a href="#">CLIC2</a>	<a href="#">1193</a>		
<a href="#">ENSG00000155961</a>	<a href="#">ENST00000369454</a>	<a href="#">RAB39B</a>	<a href="#">116442</a>		
<a href="#">ENSG00000155959</a>	<a href="#">ENST00000286428</a>	<a href="#">VBP1</a>	<a href="#">7411</a>		
<a href="#">ENSG00000214827</a>	<a href="#">ENST00000330425</a>	<a href="#">MTCP1</a>	<a href="#">4515</a>		
<a href="#">ENSG00000182712</a>	<a href="#">ENST00000369480</a>	<a href="#">MTCP1NB</a>	<a href="#">100272147</a>		
<a href="#">ENSG00000165775</a>	<a href="#">ENST00000369498</a>	<a href="#">FUND C2</a>	<a href="#">65991</a>		
<a href="#">ENSG00000197932</a>	<a href="#">ENST00000369446</a>	<a href="#">F8A1</a>	<a href="#">8263</a>		

**Result Table 1**

**Ensembl**  
Home  
Login / Register | BLAST/BLAT

New Count Results URL XML Perl Help

Dataset 105 / 49506 Genes  
Homo sapiens genes (GRCh37)  
Filters  
Chromosome: X  
Band Start: q28  
Band End: q28  
with CCDS ID(s): Only  
Attributes  
Protein  
Ensembl Gene  
Ensembl Transcript

Please select columns to be included in the output and hit 'Results' when ready

☐ Features ☐ Transcript Event  
☐ Structures ☐ Homologs  
☐ Variations ☒ Sequences

☐ SEQUENCES:  
☐ Header Information

**STEP 17:**  
To view sequences, go back to '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.

Dataset 105 / 49506 Genes  
Homo sapiens genes (GRCh37)  
Filters  
Chromosome: X  
Band Start: q28  
Band End: q28  
with CCDS ID(s): Only  
Attributes  
Ensembl Gene ID  
Ensembl Transcript ID  
cDNA sequences  
Dataset  
[None Selected]

Please select column

☐ Features ☐ Transcript Event  
☐ Structures ☐ Homologs  
☐ Variations ☒ Sequences

☐ SEQUENCES:  
Sequences (max 1)  
-----  
-----  
-----  
☐ Unspliced (Transcript)  
☐ Unspliced (Gene)  
☐ Flank (Transcript)  
☐ Flank (Gene)  
☐ Flank-coding region (Transcript)  
☐ Flank-coding region (Gene)

Upstream flank  
☐ Upstream flank

Downstream flank  
☐ Downstream flank

☐ Header Information

☐ 5' UTR  
☐ 3' UTR  
☐ Exon sequences  
☒ cDNA sequences  
☐ Coding sequence  
☐ Protein



**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 58'
- Select 'Homo sapiens genes (GRCh37)'.

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 'GENE ASSOCIATED VARIATIONS' section 'Reference ID' is selected. Also select 'Allele', 'Protein location (aa)', 'Protein Allele', and Consequence Type (Transcript Variation).

*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? These variations are not in 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/coursebook\\_58.pdf](http://www.ebi.ac.uk/~gspudich/workshop_presentations/coursebook_58.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.

Start with all the zebrafish Ensembl genes:

Choose the '**ENSEMBL Genes 58**' database.  
Choose the '**Danio rerio genes (Zv8)**' 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 1106 / 27854 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 those are available.

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

Click the [Results] button on the toolbar.

If you are happy with how the results look in the preview, output all the results:

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

### 3. Click [New].

Choose the '**ENSEMBL Genes 58**' 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).

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**' from 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 58**' 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 58**' 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 **20** out of the 23 human genes at least one mouse orthologue has been identified. ENSG00000123130 has two mouse orthologues and ENSG00000172716 has three. Three human genes (ENSG00000186594, ENSG00000130844 and ENSG00000089335) have none.

**6. Step 1:** Choose *'Ensembl Genes 58'* 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 58 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 "Variation Mart 58" 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 CASP5 (Caspase-5) 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 CASP5?

Retrieve an alignment between CASP5 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 CASP5 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)** How many within-species paralogues have been identified for this gene? Note the ‘Target %id’ and ‘Query %id’. Which paralogue has the most sequence similarity with the red-sensitive opsin?

**(c)** Have a look at the genomic location of the red-, green- and blue-sensitive opsin genes. (You should 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%)?

### Exercise 3 – The 31-Species Alignment

Find the Ensembl F9 gene for human and go to the Region in detail page.

- (a) Turn on the cow and mouse BLASTZ alignment tracks along with the frog TBLAT 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 33 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 Docs and FAQs). 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 CASP5**’ and click **[Go]**. On the page with search results click on ‘Ensembl protein\_coding Gene: **ENSG00000137757** (HGNC (curated): CASP5)’.

- (a) Click on ‘**Paralogues**’ in the side menu.

There are seven within-species paralogues predicted for human CASP5. The first one 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 CASP5 in gorilla: ENSGGOG00000015759 (CASP5).

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

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

CASP4 is related by a duplication on the level of the Simiiformes. Click on the common ancestor (red node) to see this.

- (d) Click on the duplication node (red square) or speciation node (blue square) of the 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.

There have been six within-species paralogs identified for the human red-sensitive opsin gene. Gene pairs with the highest %ID are listed at the top of the list. ENSG00000147380 (OPN1MW) and ENSG00000166160 (OPN1MW2), the genes encoding the green-sensitive (middle-wave) opsins, have the highest Target %id and Query %id.

(c) Click on '**ENSG00000166160**', '**ENSG00000147380**' and '**ENSG00000128617**'.

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 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.

## Answer Exercise 3

Under '**Search Ensembl**' type '**human gene BRCA2**' and click [Go].  
 On the page with search results click on 'Ensembl protein\_coding Gene: **ENSG00000139618 (HGNC (curated): BRCA2)**'.  
 Click on the **Location tab**.  
 Click on '**Configure this page**' in the side menu  
 Click on '**BLASTZ alignments**', select '**Cow (Bos Taurus)**' and '**Mouse (Mus musculus)**', click on '**Translated BLAT**' alignments, select '**Xenopus tropicalis**' and click [SAVE and close].

(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 exons of F9. This

reflects the Translated BLAT approach, where the genome is translated and then aligned. Alignments are focused on protein sequences.

**(b)** The pink bar corresponding to the 33 eutherian mammal alignment shows the region that could be aligned to this part of the human genome. Click on the track name to see the mammals used in this alignment. The 'Conservation score' (33 way GERP score) is calculated from this multispecies alignment. The score shows how well each nucleotide is conserved across the 33 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. (Therefore, these are the 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.

## 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?
- (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 "Resequencing 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 the grey box at the 5' terminal end of the STX7 transcripts. A pop-up box should appear, listing “ENSR00000131372” as the ID 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 (curated): PTPN22)’.

(a) Click on ‘**Variation Table**’ in the side menu.

Click on ‘**Configure this page**’ in the side menu.

Under ‘**Select Variation Type**’, deselect all options except ‘**Non-synonymous**’ and click **[SAVE and close]**.

Two of the four PTPN22 transcripts contain a SNP with amino acid (aa) change W/R and aa co-ordinate 620. This 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**’ at the left of the Variation page. The allele A is associated with Type 1 Diabetes.

##### 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 58' database.  
Choose the 'Homo sapiens genes (NCBI36)' 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)', 'Protein Allele', and 'Consequence Type (Transcript Variation)'.

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

Open in Excel.

### **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 and DNase I hypersensitive 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. These map to intronic sequence and also the 5' end of the STX7 gene (remember STX7 is a reverse-stranded gene, so its orientation is shown 3' to 5').

**(b)** Look at the “Details by cell line” link to see the core evidence is found in human cell lines K562 and CD4.

**(c)** The regulation page shows elements associated with gene regulation from multiple sources. Seven “regulatory features” are shown as shaded boxes in the diagram, and are listed in the table below. In addition, miRNA targets are indicated, from the miRanda project. CisRED motifs are also 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](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 58?

## 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. **21,701 known** and **26 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).

(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 Ensembl**' 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](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](http://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 58, CTNND1 and TMX2 are annotated as separate genes.

## **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*).