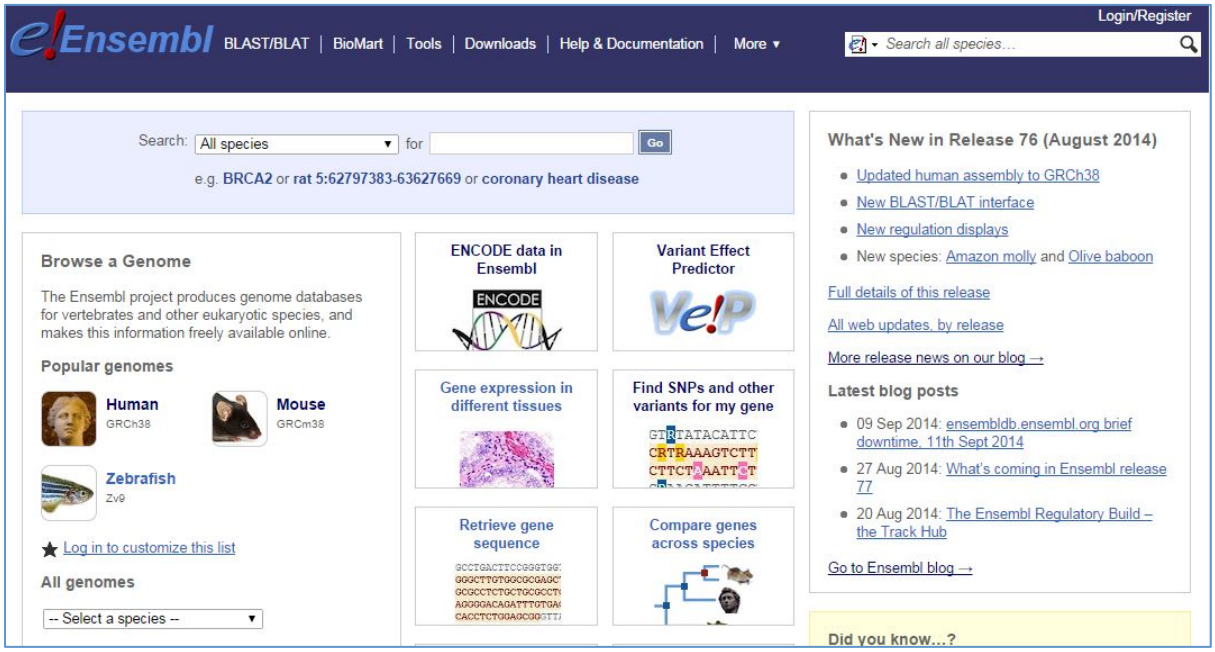
WORKED EXAMPLE – BROWSER (e76)

In this worked example we will explore the human ***ABCD1*** (ATP-binding cassette, sub-family D (ALD), member 1) gene.

The protein encoded by this gene is likely involved in the peroxisomal transport or catabolism of very long chain fatty acids (VLCFAs). Mutations in the *ABCD1* gene can cause **Adrenoleukodystrophy**, a rare X-linked disorder that causes a range of clinical phenotypes, often leading to a vegetative state and/or death (see also http://en.wikipedia.org/wiki/Adrenoleukodystrophy).

☞ Go to the Ensembl homepage (http://www.ensembl.org/).

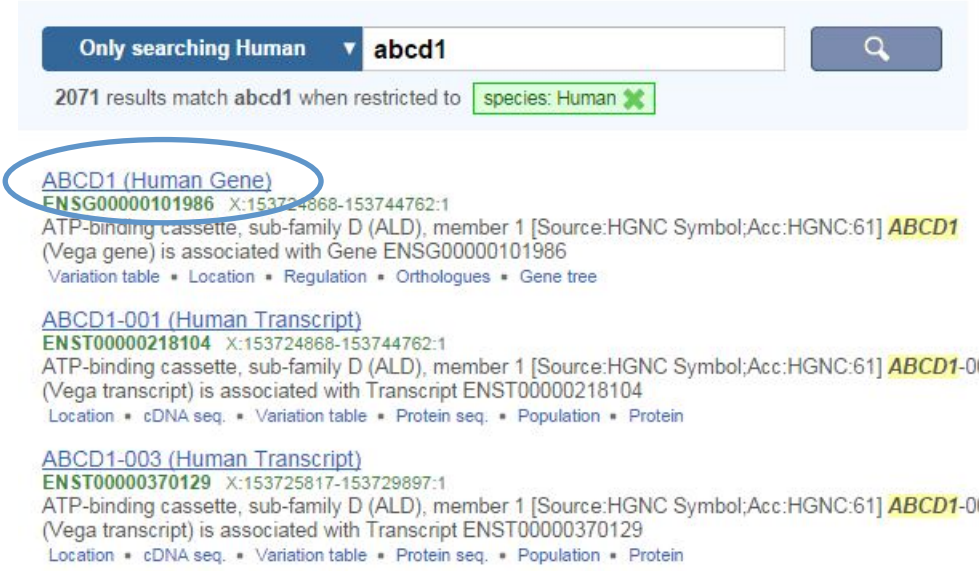


Searching

First of all, let's search for the human *ABCD1* gene.

☞ Select 'Search: Human' and type 'abcd1' in the 'for' text box.
☞ Click [Go].

The search result shows an *ABCD1* gene and several transcripts (splice variants).



Click on 'ABCD1 (Human Gene)' (the first hit)

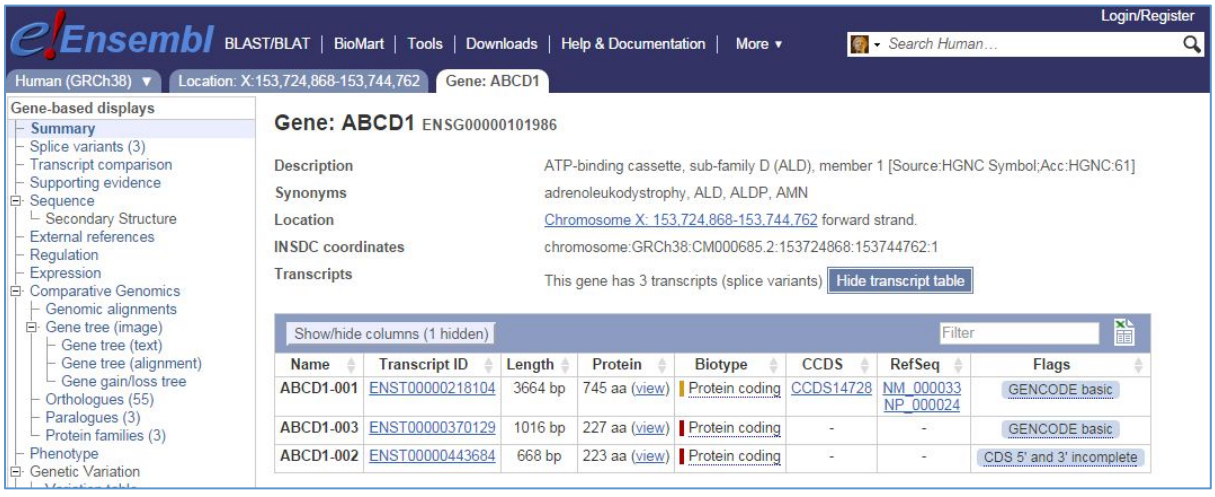
This leads us to the 'Gene summary' page under the 'Gene' tab.

The Gene tab

Pages (also called 'views') in Ensembl are organised under a number of tabs, i.e. 'Species', 'Location', 'Gene', 'Transcript', 'Variation' and 'Regulation'. The various available pages under each tab are listed in the left-hand side menu.

The 'Gene Summary' page shows general information about the *ABCD1* gene and the transcripts that have been annotated for it as part of the GENCODE gene set (http://www.gencodegenes.org/). Note the information icon (*i*) next to 'Gene summary' that opens up a help page, as well as the legend at the bottom of the graphical display.

Click [Show transcript table].



You can customise the table by clicking on 'Show/hide columns'. For example, turn on the UniProt matches, and turn off the Flags.

Looking in the table, ABCD1-001 (ENST00000218104) has a CCDS (Consensus Coding Sequence; http://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi) identifier. The CCDS is a set of coding sequences about which Ensembl, Havana, NCBI and UCSC all agree and which therefore are considered to be of high quality. In the transcript table it can be seen that two transcripts are part of the GENCODE Basic Set, which is intended to provide a simplified subset of the GENCODE transcript annotations. Transcript ABCD1-002 / ENST00000443684 is not part of this set, because its CDS is incomplete.

The graphical display shows the same three transcripts as the table. You can click on a transcript to learn more about it.

Protein-coding transcripts are gold or red. Gold transcripts are identical between the Ensembl and Havana projects, thus reflect a high standard. Red transcripts have either been annotated by Ensembl or Havana. In this case, ABCD1-002 and ABCD1-003 have been annotated by Havana.

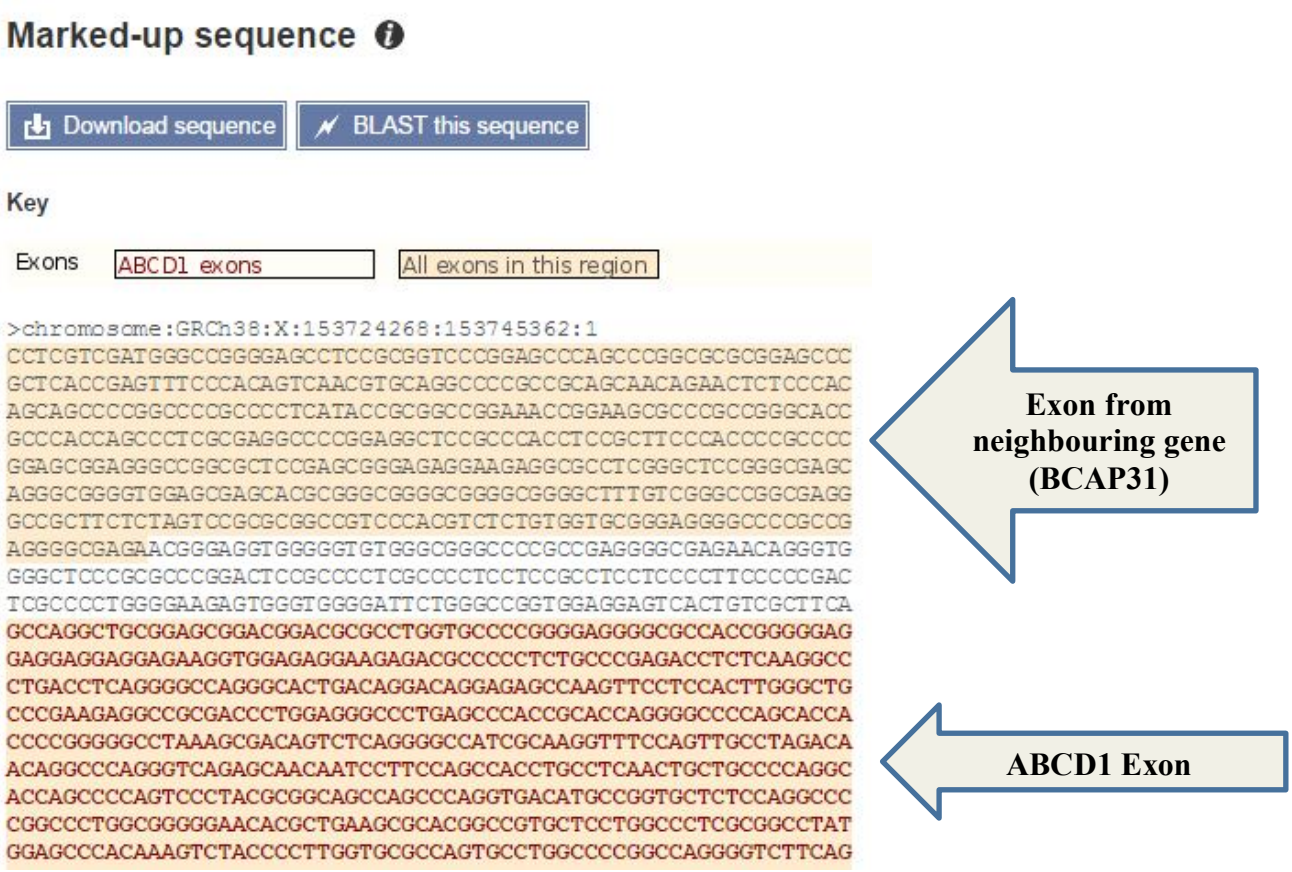
Boxes and lines in the transcripts represent exons and introns, respectively. Empty boxes represent untranslated regions (UTRs), while filled boxes represent the coding sequence (CDS).

Summary – ABCD1 Transcripts

- There are three transcripts, all protein coding
- ABCD1-001 is gold, a symbol of high quality

The *ABCD1* gene is located on the forward strand of the genome. This can be seen from the arrows next to the transcript names, which indicate the direction of transcription and from the fact that the transcript models are shown above the blue bar that represents the genome. Transcripts located on the reverse strand are shown below the blue bar.

Click on 'Sequence' in the side menu.



On the 'Sequence' page the sequence of the *ABCD1* gene plus 600 bp upstream and downstream is shown. Exon sequences belonging to the *ABCD1* gene are shown in red letters on a peach background, while exons belonging to other genes are shown in black letters on a peach background. All possible exon sequence is shown, across all the transcripts.

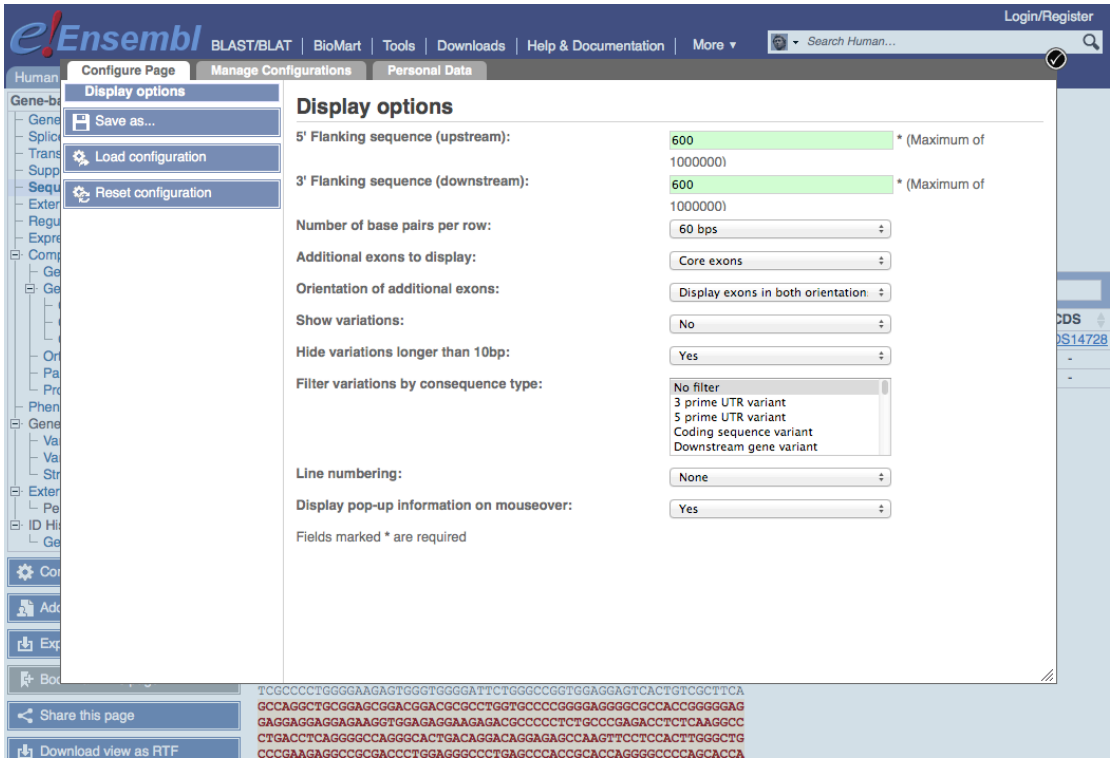
Almost all graphical displays in Ensembl can be configured. This is done using the [Configure this page] button.

Click [Configure this page] in the side menu.

A pop-up window lists all display options.

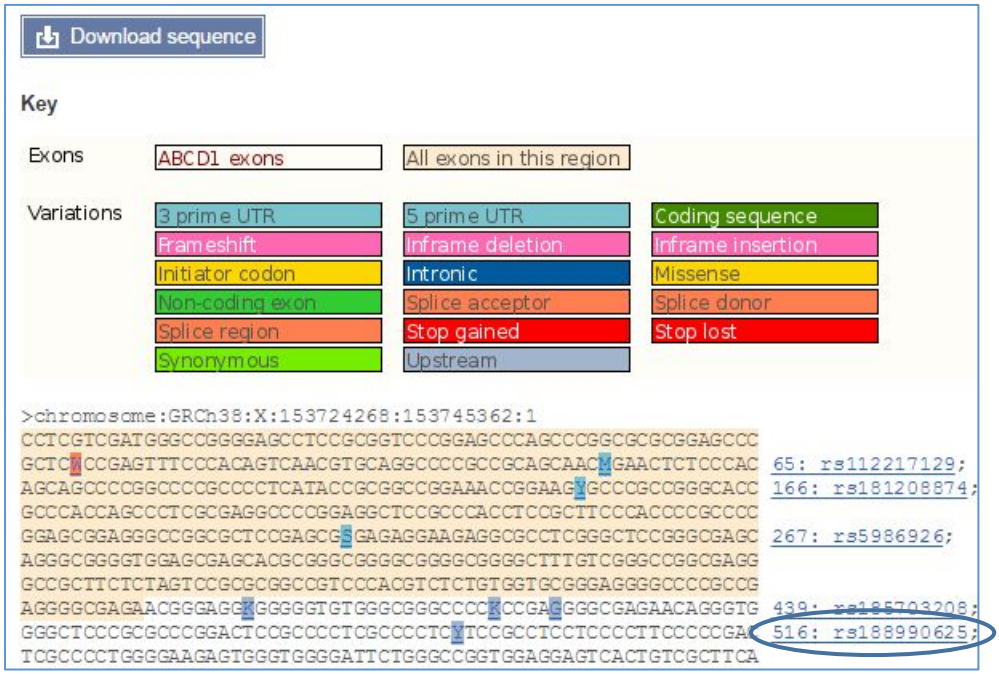
Summary – Gene Sequence

- All exons are highlighted, for all genes in the region
- ABCD1 exons are in bold letters



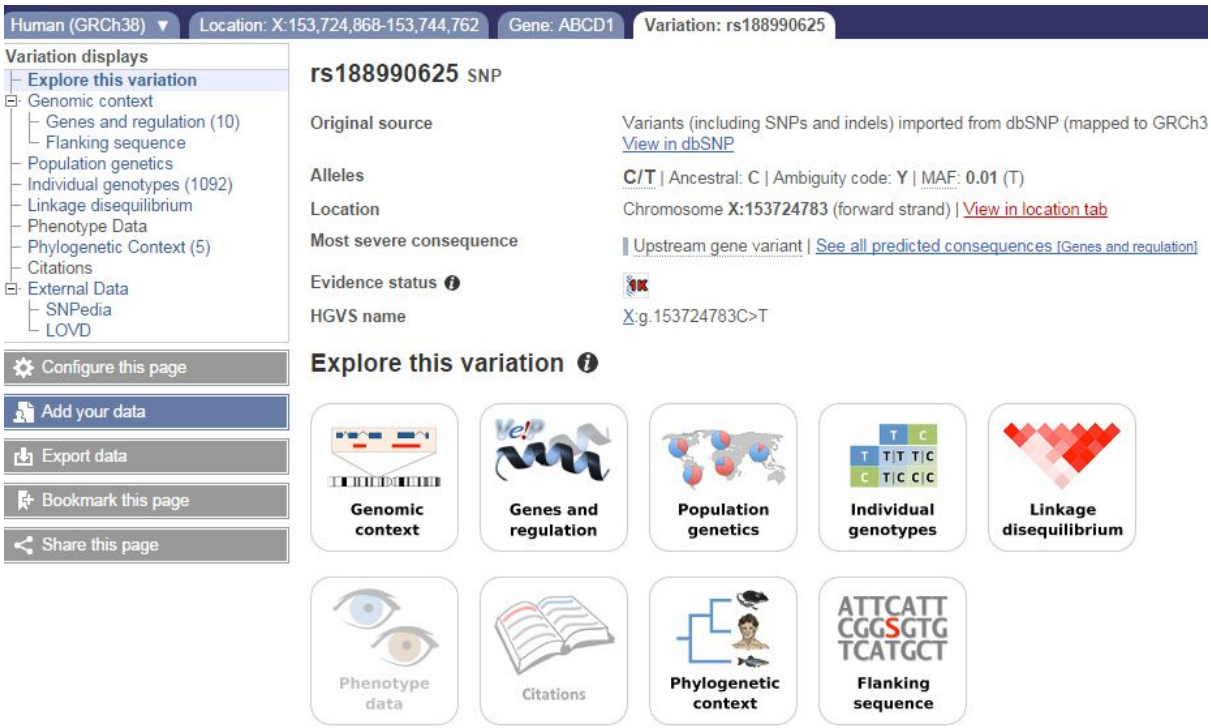
- Select 'Show variations: Yes and show links'.
- Click (✓).

The 'Sequence' page reloads, and shows the position of variants as well as links to pages where more detailed information about them can be found. The variants are shown in the sequence in IUPAC notation (http://en.wikipedia.org/wiki/Nucleic_acid_notation).



- Click on rs188990625 (circled above)

This opens the Variation tab for this sequence variant.



This is a summary page showing basic information about the variant.

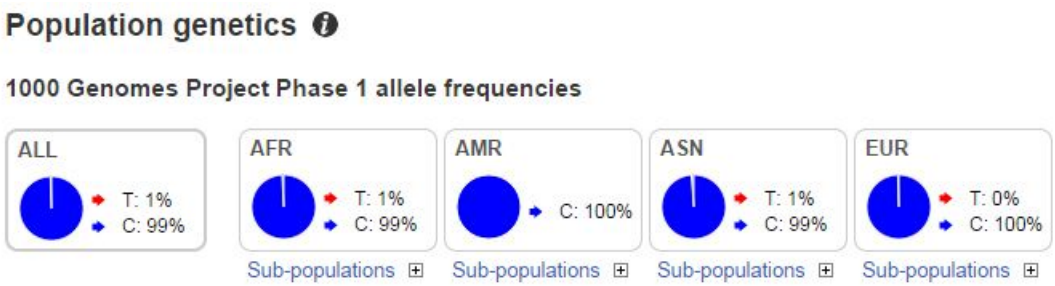
Summary – rs188990625

- It is a SNP with alleles C/T
- It's upstream of a gene
- It's found in the 1000 Genomes project

Either click on an icon or one of the left-hand links to navigate through the information for this SNP.

Click on Population genetics

Find out more about the frequency of the T and C alleles across world populations.



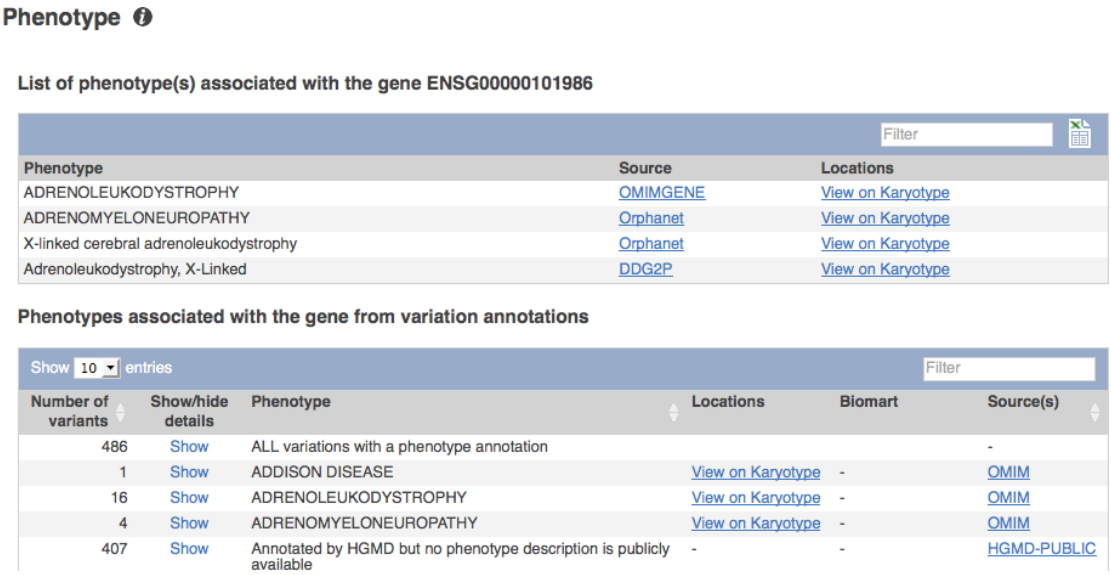
- **ALL**, All populations
- **AFR**, African
- **AMR**, Mixed American
- **ASN**, East Asian
- **EUR**, European

Expand Sub-populations for more of a break-down. This FAQ helps you interpret the population codes:

http://www.ensembl.org/Help/Faq?id=328

Click on 'Phenotype' in the side menu.

On the 'Phenotype' page phenotypes that have been associated with the *ABCD1* gene as well as with variants associated with the *ABCD1* gene are shown.



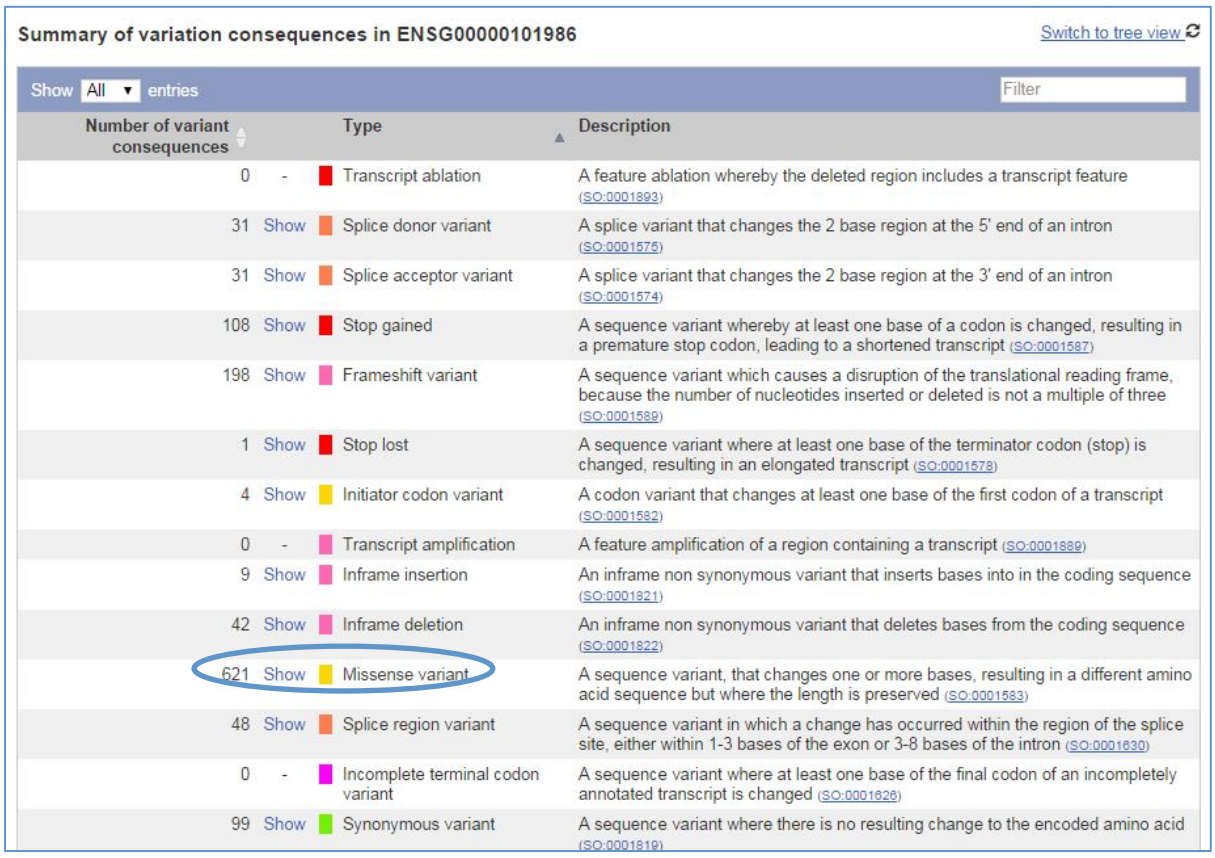
Now go back to information about the *ABCD1* gene.

Click on the Gene tab

We can see all variation for this gene.

Click on 'Genetic Variation - Variation table' in the side menu.

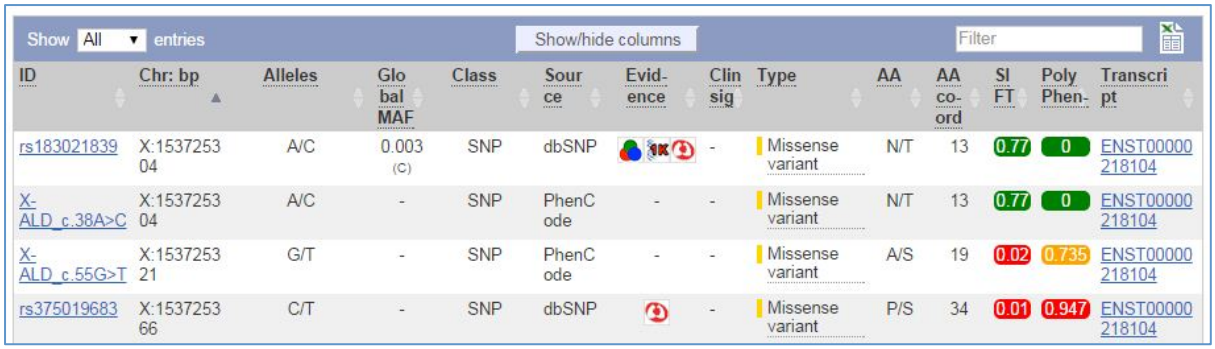
On the 'Variation table' page all variants in *ABCD1* gene are shown, grouped by consequence type.



Number of variant consequences		Type	Description
0	-	Transcript ablation	A feature ablation whereby the deleted region includes a transcript feature (SO:0001893)
31	Show	Splice donor variant	A splice variant that changes the 2 base region at the 5' end of an intron (SO:0001575)
31	Show	Splice acceptor variant	A splice variant that changes the 2 base region at the 3' end of an intron (SO:0001574)
108	Show	Stop gained	A sequence variant whereby at least one base of a codon is changed, resulting in a premature stop codon, leading to a shortened transcript (SO:0001587)
198	Show	Frameshift variant	A sequence variant which causes a disruption of the translational reading frame, because the number of nucleotides inserted or deleted is not a multiple of three (SO:0001589)
1	Show	Stop lost	A sequence variant where at least one base of the terminator codon (stop) is changed, resulting in an elongated transcript (SO:0001578)
4	Show	Initiator codon variant	A codon variant that changes at least one base of the first codon of a transcript (SO:0001582)
0	-	Transcript amplification	A feature amplification of a region containing a transcript (SO:0001889)
9	Show	Inframe insertion	An inframe non synonymous variant that inserts bases into in the coding sequence (SO:0001821)
42	Show	Inframe deletion	An inframe non synonymous variant that deletes bases from the coding sequence (SO:0001822)
621	Show	Missense variant	A sequence variant, that changes one or more bases, resulting in a different amino acid sequence but where the length is preserved (SO:0001583)
48	Show	Splice region variant	A sequence variant in which a change has occurred within the region of the splice site, either within 1-3 bases of the exon or 3-8 bases of the intron (SO:0001630)
0	-	Incomplete terminal codon variant	A sequence variant where at least one base of the final codon of an incompletely annotated transcript is changed (SO:0001626)
99	Show	Synonymous variant	A sequence variant where there is no resulting change to the encoded amino acid (SO:0001819)

Click on 'Show' for 'Missense variant'.

This results in a list of all missense variants. Clicking on the ID of a variant will lead us to the 'Variation' tab, where more information about the variant in question can be found.



Show All entries | Show/hide columns | Filter

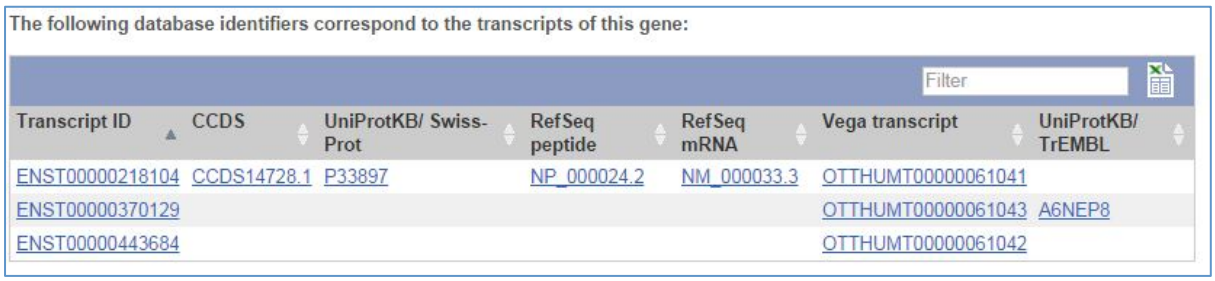
ID	Chr: bp	Alleles	Global MAF	Class	Source	Evidence	Clin sig	Type	AA	AA coord	SIFT	PolyPhen	Transcript
rs183021839	X:153725304	A/C	0.003 (C)	SNP	dbSNP		-	Missense variant	N/T	13	0.77	0	ENST00000218104
X-ALD_c.38A>C	X:153725304	A/C	-	SNP	PhenCode	-	-	Missense variant	N/T	13	0.77	0	ENST00000218104
X-ALD_c.55G>T	X:153725321	G/T	-	SNP	PhenCode	-	-	Missense variant	A/S	19	0.02	0.735	ENST00000218104
rs375019683	X:153725366	C/T	-	SNP	dbSNP		-	Missense variant	P/S	34	0.01	0.947	ENST00000218104

Clicking on a variant ID will lead us to the 'Variation' tab, where detailed information about the variant in question can be found.

Let's find out more about *ABCD1.*

Click on External References in the side menu.

This shows matches to the Ensembl gene in other projects and databases. A table that links Ensembl transcripts to UniProt and RefSeq identifiers is found at the bottom of the page.



The following database identifiers correspond to the transcripts of this gene:

Filter

Transcript ID	CCDS	UniProtKB/ Swiss-Prot	RefSeq peptide	RefSeq mRNA	Vega transcript	UniProtKB/ TrEMBL
ENST00000218104	CCDS14728.1	P33897	NP_000024.2	NM_000033.3	OTTHUMT00000061041	
ENST00000370129					OTTHUMT00000061043	A6NEP8
ENST00000443684					OTTHUMT00000061042	

This is similar to what we saw in the Transcript table, but has more information.

Click on 'Phenotype' in the side menu.

On the 'Phenotype' page phenotypes that have been associated with the *ABCD1* gene as well as with variants associated with the *ABCD1* gene are shown.

Phenotype

List of phenotype(s) associated with the gene ENSG00000101986

Filter

Phenotype	Source	Locations
Adrenoleukodystrophy, X-Linked	DDG2P	View on Karyotype
ADRENOLEUKODYSTROPHY	OMIMGENE	View on Karyotype
CADDS	Orphanet	View on Karyotype
ADRENOMYELONEUROPATHY	Orphanet	View on Karyotype
X-linked cerebral adrenoleukodystrophy	Orphanet	View on Karyotype

Phenotypes associated with the gene from variation annotations

Show 10 entries Filter

Number of variants	Show/hide details	Phenotype	Locations	Biomart	Source(s)
475	Show	ALL variations with a phenotype annotation			-
1	Show	ADRENOLEUKODYSTROPHY	View on Karyotype	-	OMIM
1	Show	ADRENOMYELONEUROPATHY	View on Karyotype	-	OMIM
410	Show	Annotated by HGMD but no phenotype description is publicly available	-	-	HGMD-PUBLIC
1	Show	COSMIC:tumour_site:NS	View on Karyotype	View list in BioMart	COSMIC
2	Show	COSMIC:tumour_site:autonomic_ganglia	View on Karyotype	View list in BioMart	COSMIC
4	Show	COSMIC:tumour_site:breast	View on Karyotype	View list in BioMart	COSMIC
1	Show	COSMIC:tumour_site:central_nervous_system	View on Karyotype	View list in BioMart	COSMIC
19	Show	COSMIC:tumour_site:endometrium	View on Karyotype	View list in BioMart	COSMIC
3	Show	COSMIC:tumour_site:haematopoietic_and_lymphoid_tissue	View on Karyotype	View list in BioMart	COSMIC

Click on 'Comparative Genomics- Orthologues' in the side menu.

All *ABCD1* orthologues in other species as identified by Ensembl are shown in the 'Selected orthologues' table on the 'Orthologues' page. Table columns can be hidden using the [Show/hide columns] button. Rows can be (re)ordered using the triangles next to the column headers and filtered using the 'Filter' text box. The table can be exported as an Excel spreadsheet by clicking on the Excel icon.

Select 'Rodents' and find the mouse orthologue

Mouse (*Mus musculus*)	1-to-1	0.06621	ENSMUSG00000031378 Abcd1 ATP-binding cassette, sub-family D (ALD), member 1 [Source:MGI Symbol;Acc:MGI:1349215]	• Region Comparison • Alignment (protein) • Alignment (cDNA) • Gene Tree (image)	X:73716597-73738534:1	90	89

For the human *ABCD1* gene, a 1-to-1 orthologue has been identified in mouse, i.e. ENSMUSG00000031378.

Summary – Human *ABCD1*

- Has three transcripts
- Has a match to UniProt P33897
- Has an orthologue in mouse
- Is linked to Adrenoleukodystrophy

Let's look specifically at one splice variant.

Click on 'ENST00000218104' in the transcript table at the top of the page.

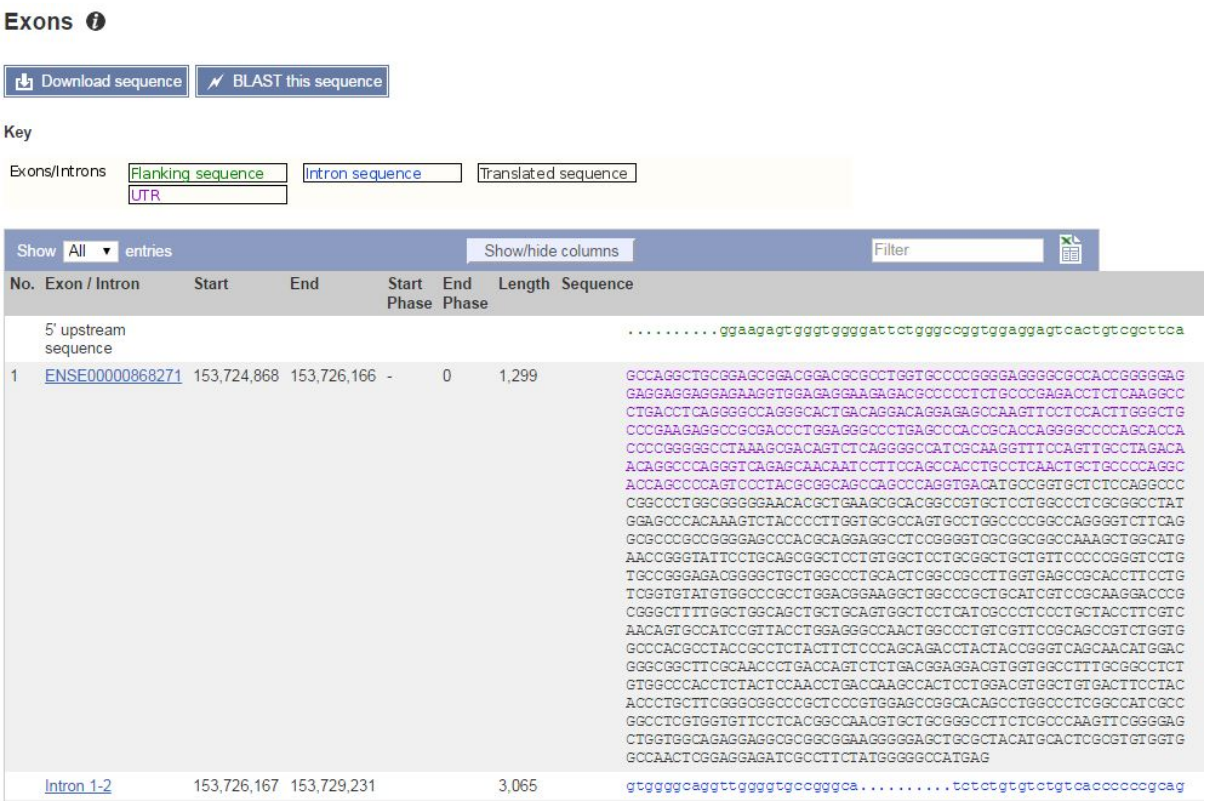
This leads us to the 'Transcript summary' page under the 'Transcript' tab.

The Transcript tab

Note that, because we have moved from the 'Gene' tab to the 'Transcript' tab, the side menu has changed and now shows links to pages with information about this specific splice variant.

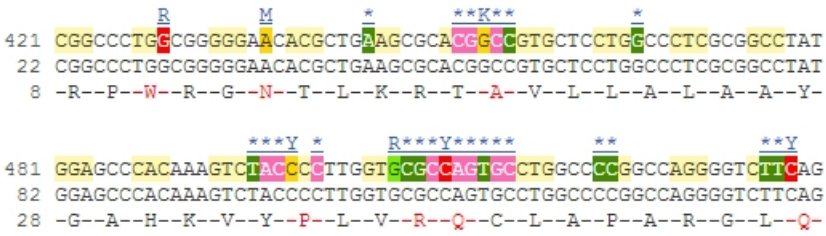
Click on 'Sequence - Exons' in the side menu.

On the 'Exons' page the sequence of the unspliced transcript is shown. The coding sequence (CDS) is shown in black, untranslated regions (UTRs) in purple, introns in blue and flanking sequences in green. By default only a small part of the introns and the flanking sequences is shown, but this can be changed on the configuration page.



Click on 'Sequence - cDNA' in the side menu.

The spliced transcript, as well as its coding sequence and translation are shown with sequence variants drawn. The page can be simplified with 'Configure this page'.



Click on 'External References - General identifiers' in the side menu.

On the 'General identifiers' page cross-references to other databases are shown that contain entries that correspond to the ENST00000218104 sequence.

General identifiers

This transcript corresponds to the following database identifiers:

Show All entries Filter

External database	Database identifier
CCDS	CCDS14728.1 [view all locations]
European Nucleotide Archive	BC015541 [align] [view all locations] BC025358 [align] [view all locations] U52111 [align] [view all locations] Z21876 [align] [view all locations] Z31006 [align] [view all locations] Z31007 [align] [view all locations] Z31008 [align] [view all locations] Z31009 [align] [view all locations] Z31010 [align] [view all locations] Z31348 [align] [view all locations]
HGNC transcript name	ABCD1-001 ATP-binding cassette, sub-family D (ALD), member 1 [view all locations]
Havana translation	OTTHUMP00000025960 [view all locations]
Human Protein Atlas	HPA035214 [view all locations] HPA035214 [view all locations]
INSDC protein ID	AAH15541.1 [align] [view all locations] AAH25358.1 [align] [view all locations] CAA79922.1 [align] [view all locations] CAA83230.1 [align] [view all locations]
RefSeq mRNA	NM_000033.3 [align] [view all locations]
RefSeq peptide	NP_000024.2 [Target %id: 100; Query %id: 100] [align] ATP-binding cassette sub-family D member 1 [view all locations]
UCSC Stable ID	uc004fif.2 [view all locations]
UniParc	UPI0000000DF5 [view all locations]
UniProtKB/Swiss-Prot	P33897 [align] ATP-binding cassette sub-family D member 1 [view all locations]

For example, ENST00000218104 matches the P33897 protein sequence in the UniProtKB/Swiss-Prot database (http://www.uniprot.org/) and the NP_000024.2 protein and NM_000033.3 mRNA sequence in the RefSeq database (http://www.ncbi.nlm.nih.gov/refseq/).

Click on 'Ontology - GO table' in the side menu.

Gene Ontology (GO) terms (http://www.geneontology.org) associate proteins to biological process, molecular function and cellular component terms.

GO table

- GO: Biological process
- GO: Cellular component
- GO: Molecular function

Descendants of GO: Biological process

Show All entries | Show/hide columns | Filter

Accession	Term	Evidence	Annotation Source	GOSlim Accessions	GOSlim Terms
GO:0006200	ATP catabolic process	IDA		GO:0034641 GO:0034655 GO:0009056 GO:0008150 GO:0044281	cellular nitrogen compound metabolic process nucleobase-containing compound catabolic process catabolic process biological_process small molecule metabolic process
GO:0006635	fatty acid beta-oxidation	IDA		GO:0009056 GO:0008150 GO:0044281 GO:0006629	catabolic process biological_process small molecule metabolic process lipid metabolic process
GO:0006810	transport	IEA	InterPro:FA_transporter	GO:0008150	biological_process
GO:0007031	peroxisome organization	NAS		GO:0008150	biological_process

The ‘biological process’ terms indicate that the ABCD1 protein plays a role in fatty acid transport and catabolism. The ‘cellular component’ terms indicate the ABCD1 protein is located in the peroxisomal membrane.

Let’s have a look at the region on the chromosome, and neighbouring genes.

Click on the 'Location' tab.

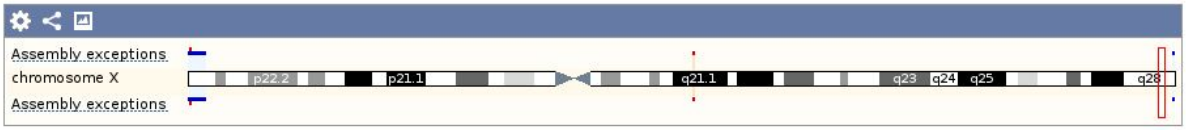
This leads us to the ‘Region in detail’ page under the ‘Location’ tab.

The Location tab

The ‘Region in detail’ page shows the genomic neighbourhood of the *ABCD1* gene. It consists of three parts.

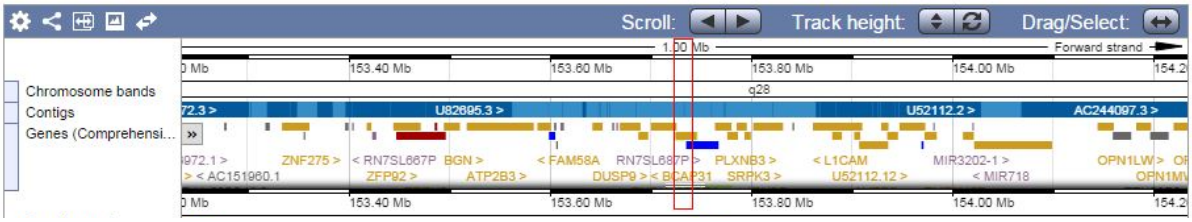
First, the complete chromosome.

Chromosome X: 153,724,868-153,744,762

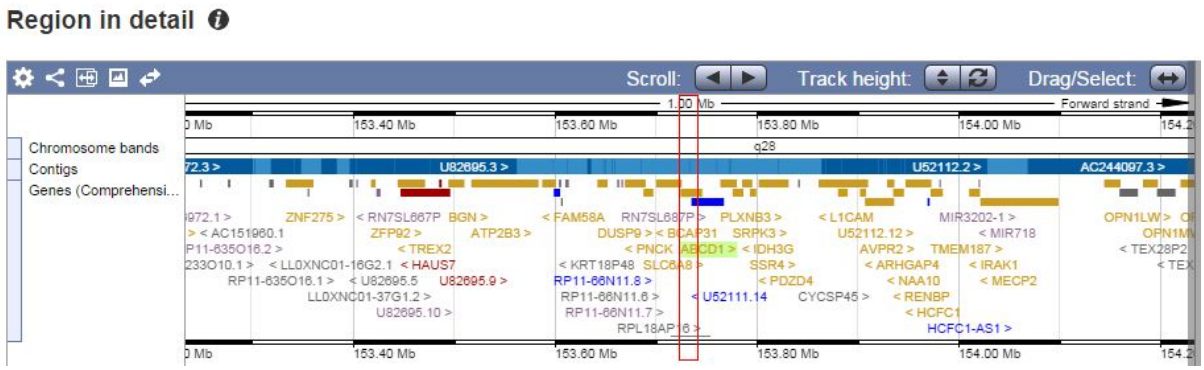


Second, the 1 Mb region around the gene of interest.

Region in detail



Drag down the bottom to reveal *ABCD1*.



This display is scrollable. Either use the ‘Scroll’ arrows click and drag the image in the same way as Google Maps. Zoom in by clicking the ‘Drag/Select’ icon, selecting the region of interest with your mouse and subsequently clicking ‘Jump to region’ in the resulting pop-up.

Third, the region of interest. In our case this is the *ABCD1* gene.

By default, the data tracks drawn are:

- 38 way GERP elements (the ‘constrained elements’, which are regions of high conservation based on comparison of sequence across 38 species)
- Human cDNAs (cDNA sequences aligned to the genome)
- CCDS set (transcripts in the Consensus Coding Sequence Set)
- Genes (GENCODE)

- Contigs (the genome)
- 1KG-All-common (Variants from the 1000 Genomes project with population frequency >1%)
- All phenotype-associated variants
- 1000 Genomes High Quality Structural Variants
- MultiCell regulatory features (sequences that may be involved in gene regulation)
- %GC (reflects GC content vs AT)

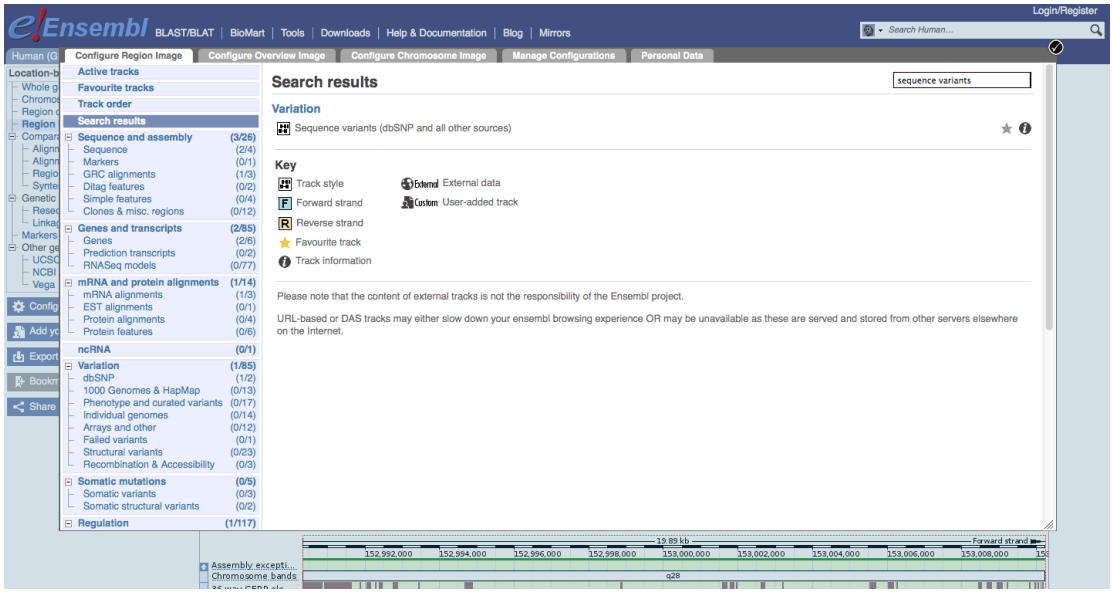
There are several ways to navigate this display:

- zoom in and out by using the [+/-] slider
- zoom in by drawing a box around the region of interest and subsequently clicking 'Jump to region' in the resulting pop-up
- moving up- and downstream with the single and double arrows next to the [+/-] slider.
- going to a particular region by changing the coordinates in the 'Location' text box or by searching for a gene using the 'Gene' text box (which has auto completion)

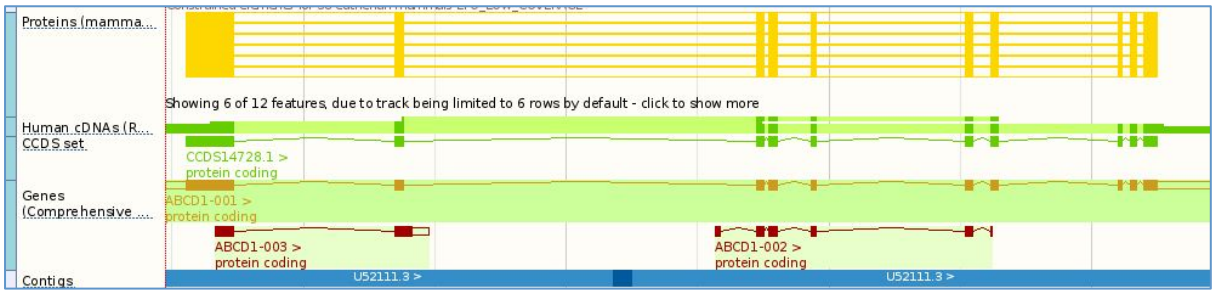
Datasets (or tracks) can be added to the display using [Configure this page]. On the configuration page all available tracks are grouped in the left-hand menu. It is also possible to search for tracks using the 'Find a track' text box.

For example, to add protein alignments from UniProt to the display:

- Click [Configure this page] in the side menu.
- Type 'UniProt in the 'Find a track' text box.
- Select 'Proteins (mammal) from UniProt'. Choose 'Normal'.
- Click (✓).



A new track, 'UniProt (mammals)', has now been added to the display.



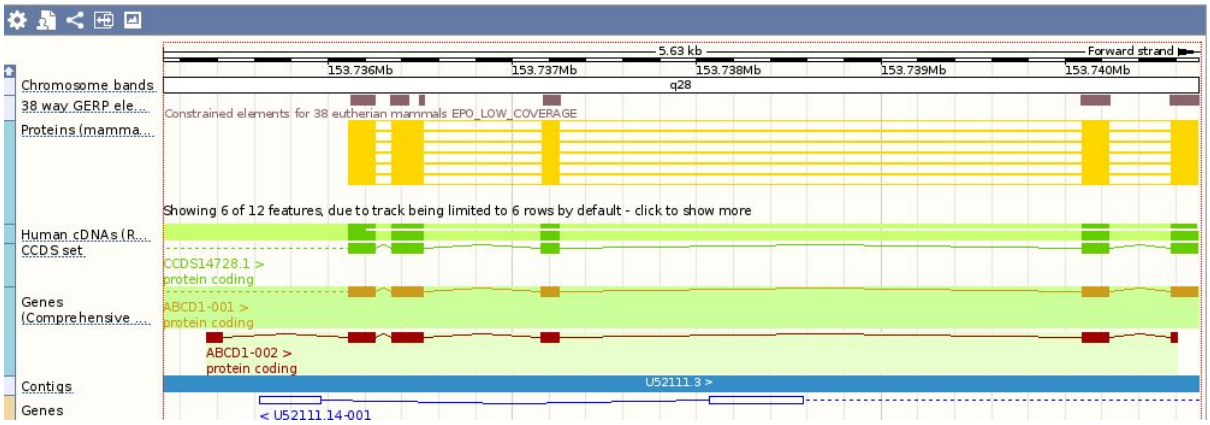
To turn the added track off again:

- Hover over the track name.
- Click on the 'Turn track off' icon (x) in the pop-up.

Tracks can be moved by clicking on the bar in front of the track name and dragging the track to the desired location.

To zoom in, you can click and drag your mouse around a region.

- Zoom in to ABCD1-002



At the top of the display (circled in the image above) several icons are shown, some of which can also be found on other displays:

- Configure this image: add/delete tracks (same as [Configure this page] button in the side menu).
- Manage your custom tracks: add your own data (same as [Add your data] button in the side menu)
- Share this image: create a URL that can be shared with others without the need to tell them how to configure the page
- Resize this image: resize the image
- Export this image: export the image in various formats (PDF, PNG etc.)

BLAST/BLAT

Finally, we will do a sequence search. For this we need some sequence. To this end, we will take the sequence of the region we are looking at.

- Click [Export data] in the side menu.
- Click [Next>].
- Click on 'Text'.

This gives us sequence in FASTA format.

```
>X dna:chromosome chromosome:GRCh38:X:153735103:153740728:1
TCCTTTTCATGGCTGAATAATATTCCATTGTGTGCATAGACCACAATTTCTTTATCCATT
CATCCCTTGATGGACATTTTGGGTTTCTTCATGTTTTGGCTATTGTGAATAACACTGCTG
TGAACATCCATGGACAAGTCTCTATGTGTGCAGATATTTTCGTTTCTCCTGGGTGTGTAG
CTAGGAGTAGAATTGCCAGGTCACATGGTAACTGGACGTTTCACTTTTTGAGGAGCTGCG
AGACTGTTCTCCACAGTGGCTGCCCCATTTTACCTTCCCGCCAGCAGTGTTGGAGGGTTC
CACCTTTTCATCGTGGCTAGCACTGGTTATCATCTCCTTTGTATTCTAGCCACCTAGTGG
GTGTGAGGCAGTATCTCTTGGTGGTTTTGATTTGCATTTCCCTGATGACTAATGACGCTG
AGCCTCTTTTGATGTGTTGAGTGGCCATTTGTATGTCTTCTTTGGAGAAATGTCTGTTCA
CGTCCTTCGCCCATGTGTGATCGGGTTATCTCTGTCGCTGAGTTGTAAAAGCTCTTTGTA
TATTCTGGATACTGCACCCTCATCAGATGTGTGGTTCACCAGTCCAGAGTTTTCTCCCAG
```

- Select and copy the sequence.
- Go back to the browser.
- Click on the 'BLAST/BLAT' link on the toolbar at the top of the page.
- Paste the sequence in the 'sequence data' text box.
- Select 'Homo_sapiens' as the species to search against.
- Select 'BLAT' as the search tool.

We use BLAT because we expect an exact match (as a rule of thumb use BLAT when you are searching against the same species your query sequence is from and use BLAST when you are searching against another species).

- Click [RUN>].
- Click [View results].

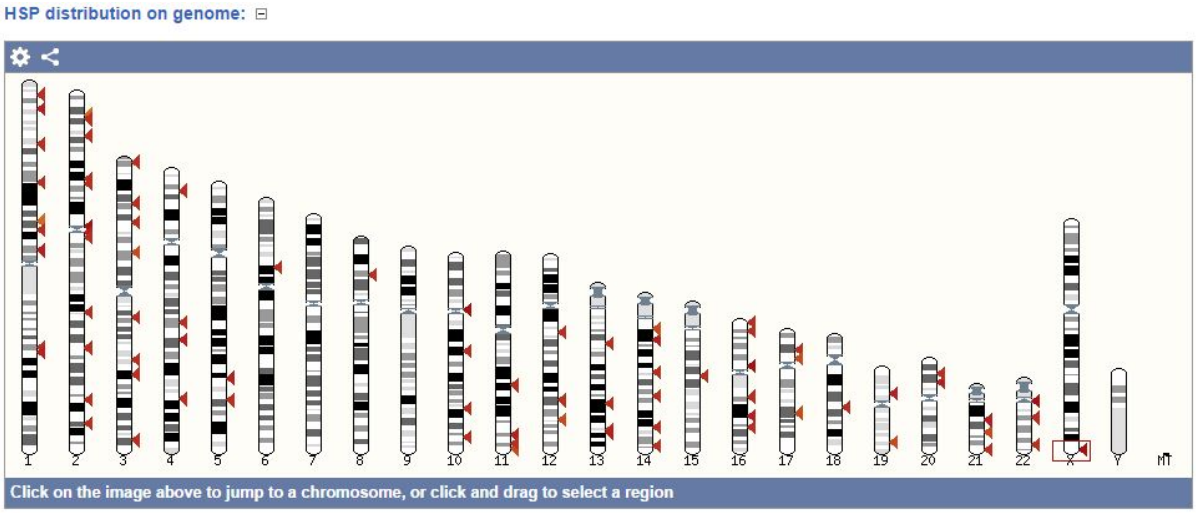
Results table:

Show All entries | Show/hide columns | Filter

Genomic Location	Orientation	Query name	Query start	Query end	Query ori	Length	Score	E-val	%ID
X:153735103-153740728 [Sequence]	Forward	X [Sequence]	1	5626	Forward	5626	11096.0	0.0e+00	100.00 [Alignment]
X:154281643-154282028 [Sequence]	Forward	X [Sequence]	3195	3580	Forward	386	699.0	1.0e-201	95.08 [Alignment]
X:154244522-154244907 [Sequence]	Forward	X [Sequence]	3195	3580	Forward	386	699.0	1.0e-201	95.08 [Alignment]
X:154206713-154207098 [Sequence]	Forward	X [Sequence]	3195	3580	Forward	386	699.0	1.0e-201	95.08 [Alignment]
X:154169585-154169970 [Sequence]	Forward	X [Sequence]	3195	3580	Forward	386	699.0	1.0e-201	95.08 [Alignment]
16:32478096-32478329 [Sequence]	Reverse	X [Sequence]	5393	5626	Forward	234	443.0	1.2e-124	96.58 [Alignment]
KI270728.1:624428-624661 [Sequence]	Forward	X [Sequence]	5393	5626	Forward	234	440.0	8.0e-124	96.15 [Alignment]

This shows all hits: the length of the match, score, e-value and %ID (% identity).

Scroll down past the results table to find the BLAST matches drawn graphically on chromosomes. The best match has a box around it.



Look at the table again. The first hit is the best one in the table (the length matches the full query sequence, the e-value is 0, and the identity is 100%).

Genomic Location	Orientation	Query name	Query start	Query end	Query ori	Length	Score	E-val	%ID
X:153735103-153740728 [Sequence]	Forward	X [Sequence]	1	5626	Forward	5626	11096.0	0.0e+00	100.00 [Alignment]
X:154281643-154282028 [Sequence]	Forward	X [Sequence]	3195	3580	Forward	386	699.0	1.0e-201	95.08 [Alignment]
X:154244522-154244907 [Sequence]	Forward	X [Sequence]	3195	3580	Forward	386	699.0	1.0e-201	95.08 [Alignment]

Click on the location of the match (circled above).

This leads us back to the 'Region in detail' page, to which now a new track named 'BLAT/BLAST hits' has been added, which shows the best hit in red.