A UCI TMF TUTORIAL: FINDING TARGETING VECTORS AND DOWNLOADING SEQUENCE FOR A MUTANT ALLELE OF YOUR GENE OF INTEREST VIA THE INTERNATIONAL KNOCKOUT MOUSE CONSORTIUM (IKMC) MARTSEARCH WEB PAGE

Enter the name of your gene of interest here – e.g. in this case “Fndc3a”

Viewing IKMC Data in Ensembl

Most of the products/data found within this portal can also be viewed as DAS tracks in the Ensembl genome browser. Follow these links to have the tracks automatically activated for you:

- IKMC alleles in Ensembl Mouse
- IKMC alleles on orthologous genes in Ensembl Human

Federated Searches

The links below take you to the standard MartView interface for several examples of federated queries:

1. Find all IKMC targeted ES cells for genes encoding transcription factors on Chromosome 1. (This query joins IKMC Projects/Alleles to Ensembl).
2. Find all IKMC mice available from the EMMA Repository with information on the vector used to make the mutation. (This query joins IKMC Targeting Repository to Mouse Production data).
3. Find all IKMC targeted ES cells for genes expressed in heart. (This query joins IKMC Projects/Alleles to EuExpress).

The Biomarts

This portal integrates information on IKMC mouse knockout resources with numerous other relevant datasets, including Ensembl, Europhenome, EuExpress and EMMA. For more information about this portal and the way in which it unites and searches the data, please see the about page.

Information for Developers

All the code and data that we produce is open-source and free to use. The following links will guide you to our source-code and documentation on how you can interact with the services we provide.

- All the code used to create this portal
- The ruby API used to interact with the Biomarts
- Using our search engine in your application
The results page

Click here to view the details about the mutant alleles generated.

In this case, both targeting vectors (DNA) and ES cells with a targeted mutation are available for purchase.
Currently, two types of targeted alleles are available. Both alleles are “mutant first”; i.e. the presence of the [splice acceptor – IRES- lacZ – poly A] cassette may result in a truncated, non-functional protein product. The locus can be reverted to a nearly wildtype sequence by expression of Flp recombinase, which will excise sequences between the Frt sites. In the case of the “conditional potential” allele, this will leave one or more exons of the gene flanked by twoloxP sites (“floxed”). Expression of cre recombinase should cause deletion of the (in this example) two floxed exons. The location of theloxP sites is designed to generate a frame-shift mutation that should result in degradation of any remaining mRNA via nonsense-mediated decay (NMD) systems.
Details of the predicted mutant allele in GenBank format

The information is in GenBank format. Before we can view and annotate this data using Lasergene’s “SeqBuilder” application, we need to reformat the information.
Copy and paste the information from your browser into a suitable text editor application – e.g. MS-Word

First, “select-all”, then “copy”
Copy and paste the information from your browser into a suitable text editor application – e.g. MS-Word

Use the “Paste Special…..” function to paste your text
Copy and paste the information from your browser into a suitable text editor application – e.g. MS-Word

Select “Unformatted Text” when pasting your text
To enable the Lasergene suite’s “EditSeq” application to import your data, save the GenBank-formatted information as a text (.txt) file.

To save your Genbank information in a format that Lasergene’s EditSeq application can recognize, select “Save As...” then select “.txt” (Text) format.
To enable the Lasergene suite’s “EditSeq” application to import your data, save the GenBank-formatted information as a text (.txt) file.

We’ve given the file an appropriate name – i.e. “Fndc3a_cKO”.

Selecting “Plain Text (.txt)” as the format produces the “.txt” file extension.
To enable the Lasergene suite’s “EditSeq” application to import your data, save the GenBank-formatted information as a text (.txt) file.

MS-Word prompts you for a format for text-encoding during the “File Conversion”. Use the default setting (“Mac OS”).

Click “OK”
Next, import your saved “.txt” file into Lasergene suite’s “EditSeq” application.

You’ve launched and are in “EditSeq”.

Use “Import” in the File menu.

Select your “.txt” file.

Select your “.txt” file.
The GenBank formatted information viewed following import into Lasergene suite’s “EditSeq” application

Your data has been imported into EditSeq

The GenBank format header information is located in this field

Each of the “features” in the predicted targeted allele are located in this field
To view the information in Lasergene’s “SeqBuilder” application, first save the information in an EditSeq (”.seq”) file format.
To view the information in Lasergene’s “SeqBuilder” application, tell SeqBuilder to open the EditSeq (".seq") file.

You are now using “SeqBuilder”

Using “File” -> “Open”, select your EditSeq (".seq") formatted file.
Your sequence information viewed in Lasergene’s “SeqBuilder” application. Remember to save the information in a SeqBuilder (“.sbd”) file format.

You are now using “SeqBuilder”

Annotated sequence information of predicted targeted allele

Features in your file