

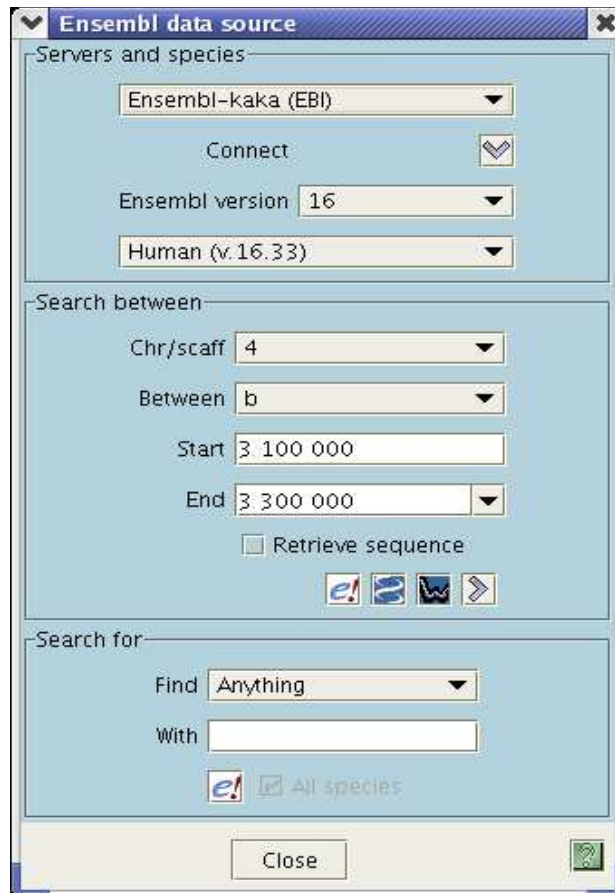
Sockeye How-to: Using Sockeye to Perform Multiple Alignments

Author: Stephen Montgomery

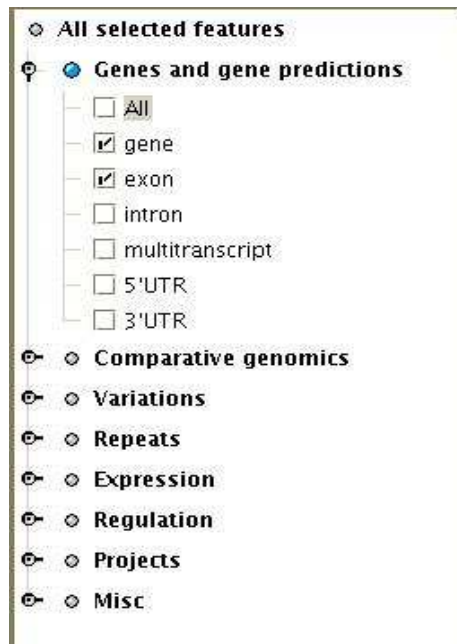
Date: August 18th, 2003

Sockeye allows a user to perform integrated alignments in a 3D environment. As of yet, the underlying alignments are not available for export but Sockeye allows users to view the gaps across multiple sequences using ClustalW or LAGAN. This document provides step-by-step instructions to observing these alignments.

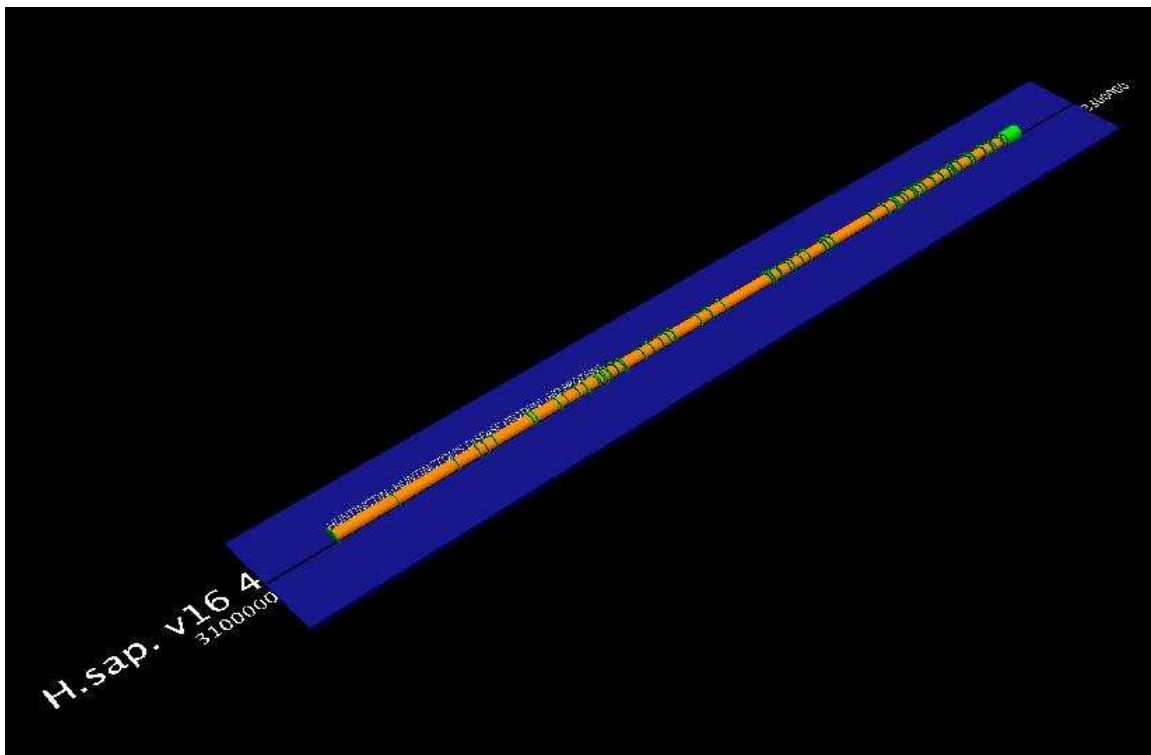
- 1) Run your version of Sockeye (1.0 or later)
- 2) Query coordinates from Ensembl (go to menu, Data -> Query Ensembl Server). Here, we have queried in the coordinates for the Huntington's disease protein in human.



3) Once the query returns, you should be able to see the Huntington's disease protein. If not, verify that genes and exons are selected in the Feature Selection Tree



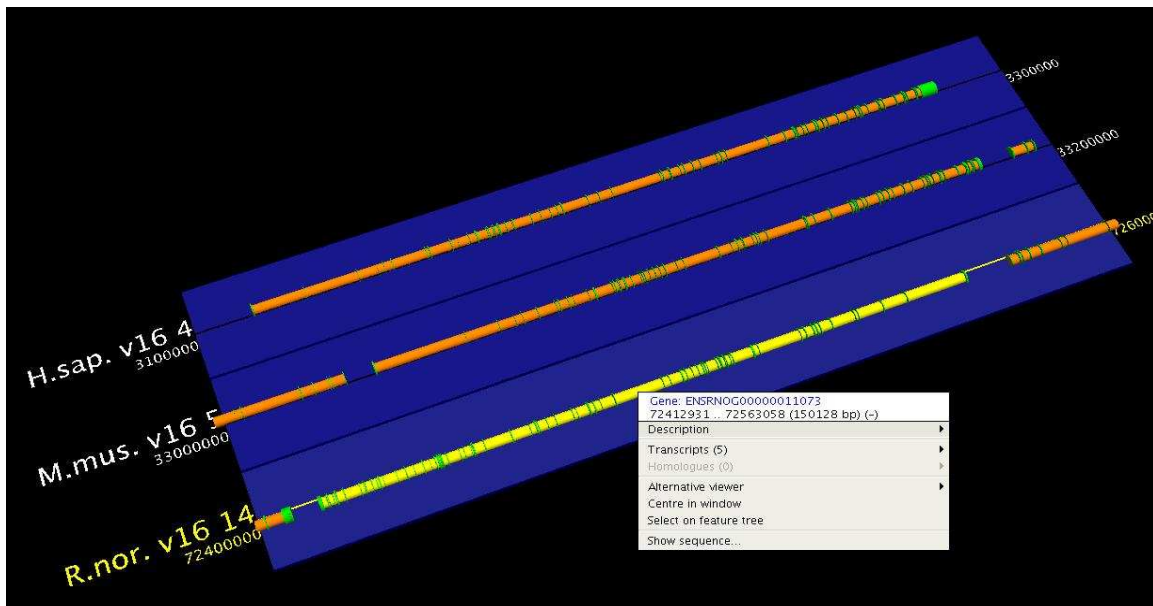
4) This is the gene in Sockeye (from EnSEMBL version 16).



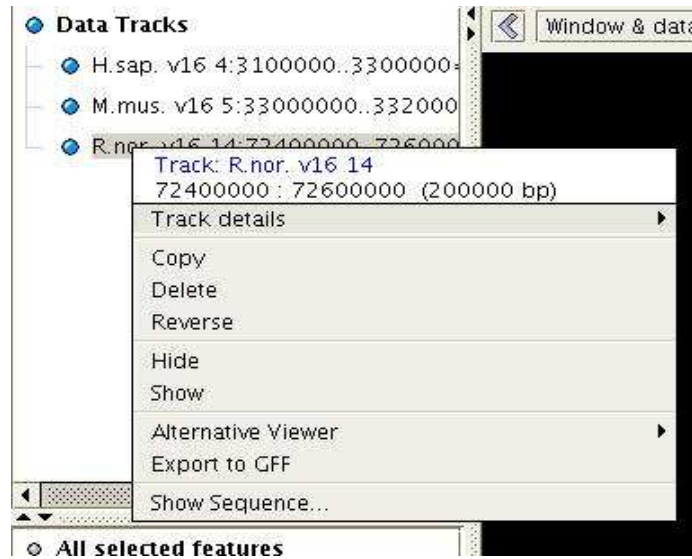
- 5) Sockeye offers an easy way to load Ensembl-curated orthologues. Right-click on your gene to get the information pop-up. (NOTE: Orthologues here says 0 but in your version of Sockeye it will say 4 and you will have the option of getting all orthologues. - This is due to a small bug – Alternately, you can query in the orthologues if you know the coordinates)



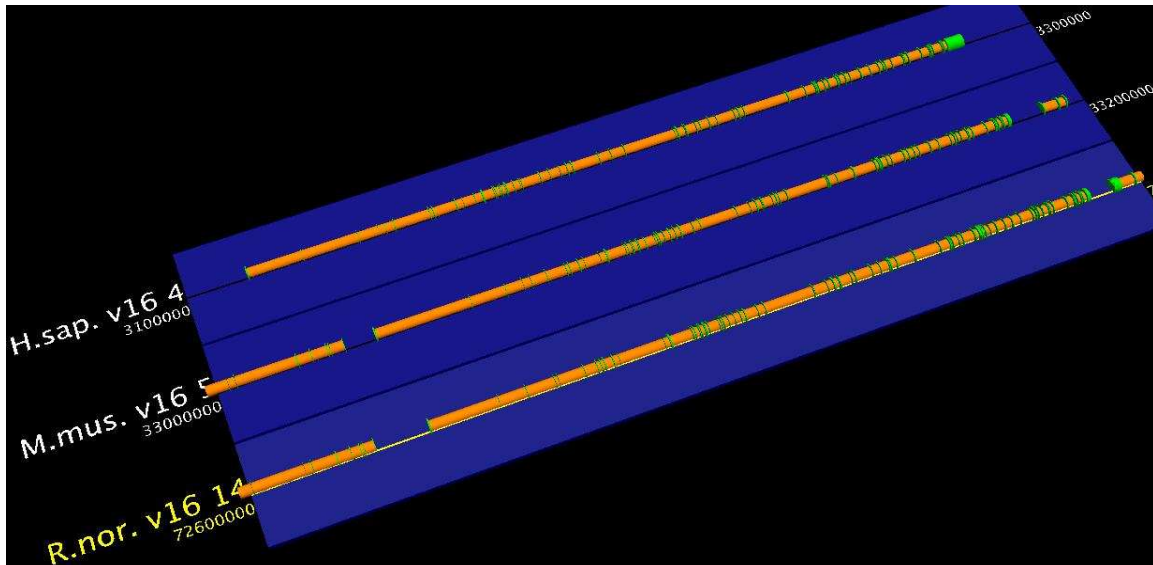
- 6) You should see something like this if you load the mouse and rat sequences. Here we have right-clicked to show that the Rat gene (on the bottom) is on the reverse strand. You can also tell this information since the gene is below the centreline (yellow line that runs through the track).



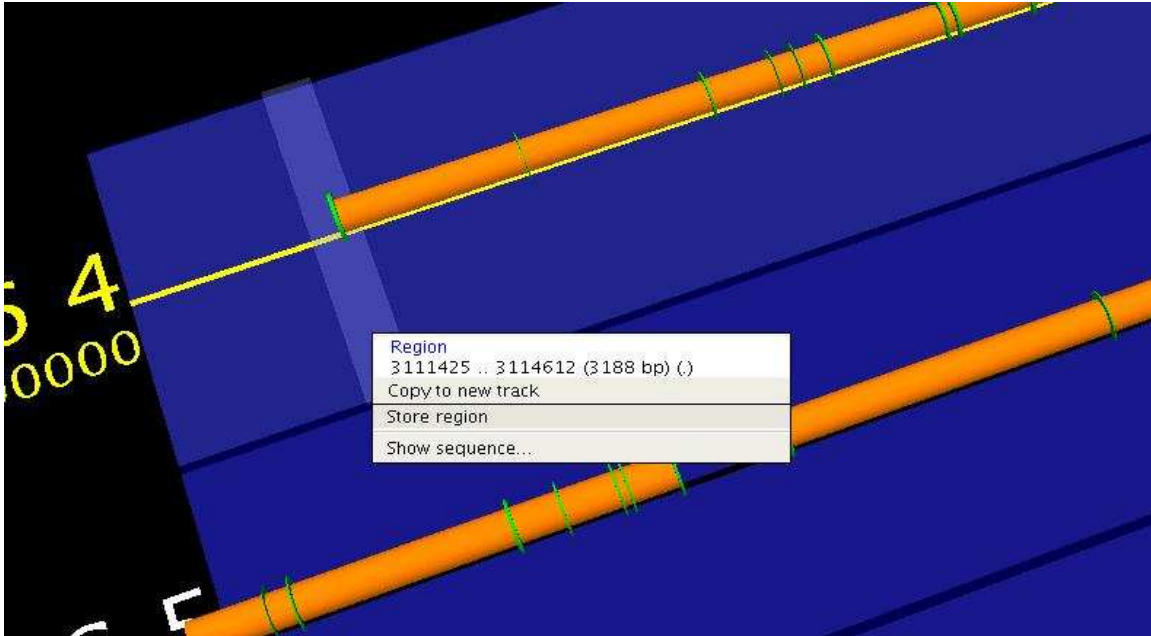
- 7) If a gene is on a reverse strand, you can visualize them side-by-side by using the reverse function in the sequence track selection tree. (Reverse is halfway down this menu)



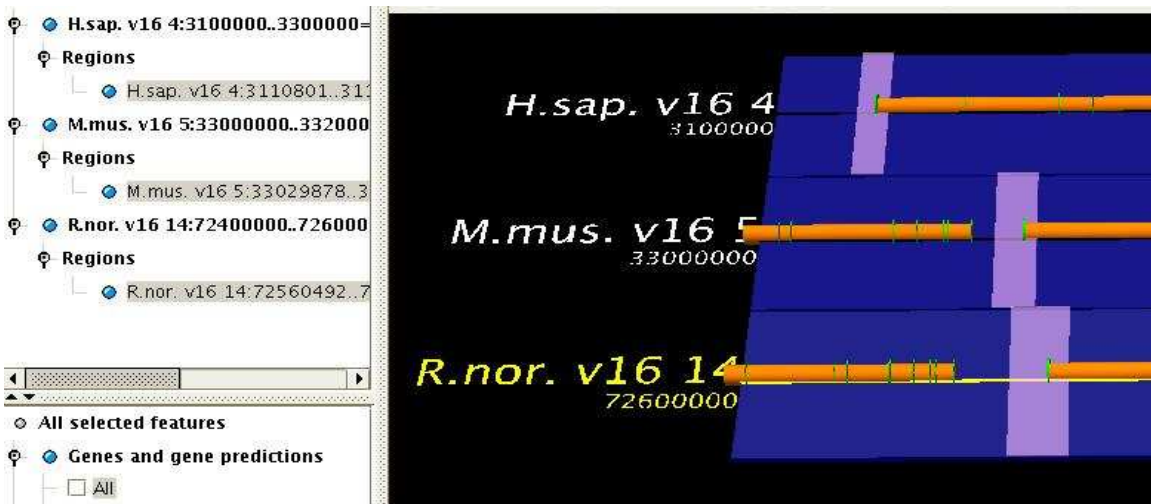
- 8) By selecting reverse, you would see this picture.



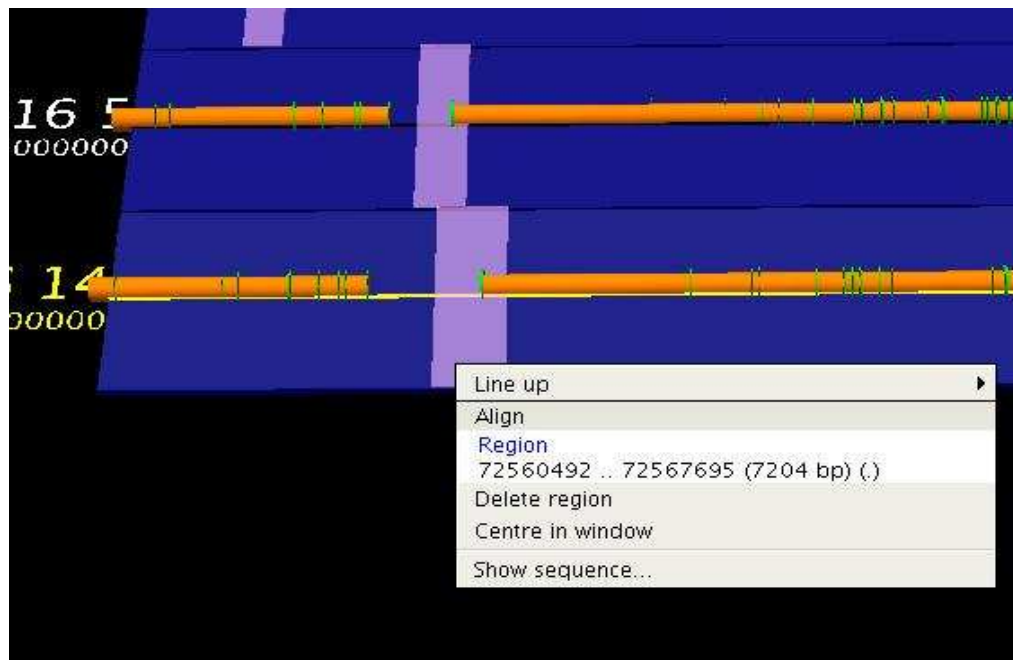
9) So how to you do a multiple alignment? The first step once you have loaded sequence tracks is to store (select regions) for alignment. This is done by clicking on a track and dragging. Once you have highlighted a region. Right-click on it a select “store region”



10) Do this for all the sequence tracks. And Left-click the tracks in the 3D viewport or on the sequence track selection tree to select them all (the selected regions will be pink)



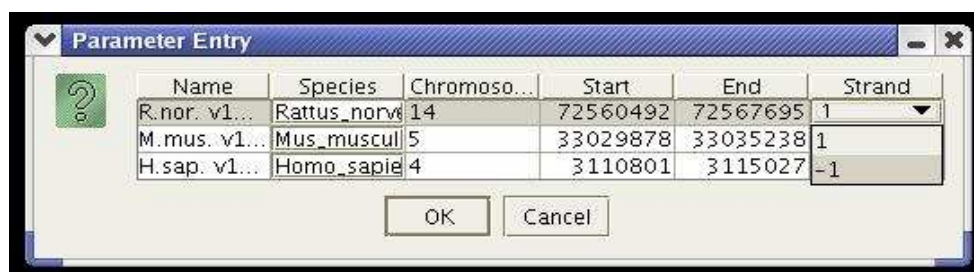
11) Right click on a highlighted region and click align.



12) You will now be able to select which alignment algorithm you want to use.



13) The sequences that you have selected for alignment will appear. Remember that rat was on a different strand, we have to specify that here.



14) The parameters for entry will appear. Red will mean that the parameter you have entered is likely wrong. Green means you are okay. (Don't worry if you see red as it isn't overly accurate for most cases but is a helpful guide).



15) The alignment results will be fired off to the Chinook server (either your own version, the default is ours). You will receive the alignment as tracks as soon as the job is finished. Below you see the gray structure (gray blocks). Congratulations!

