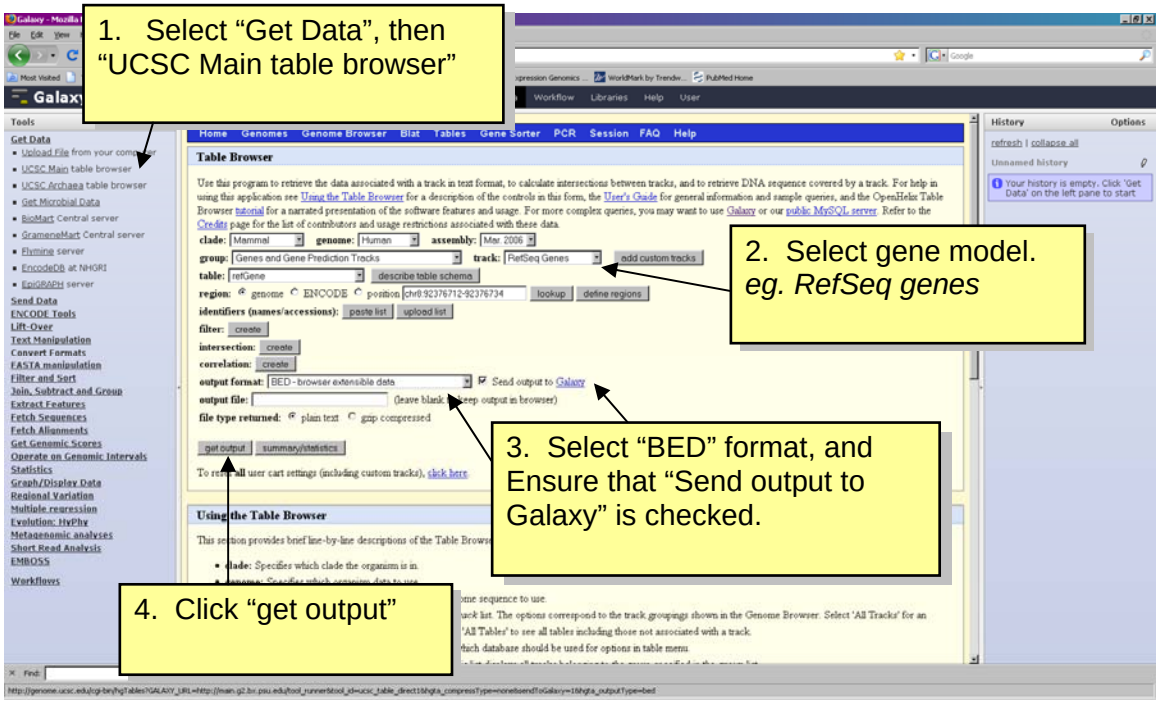
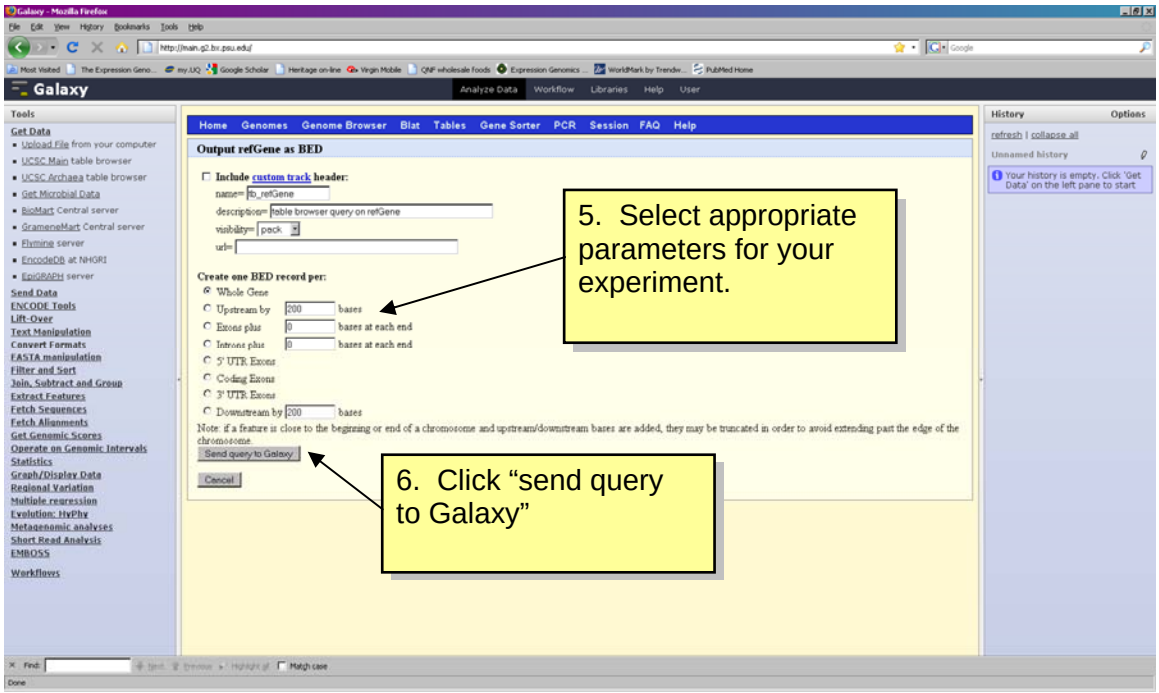
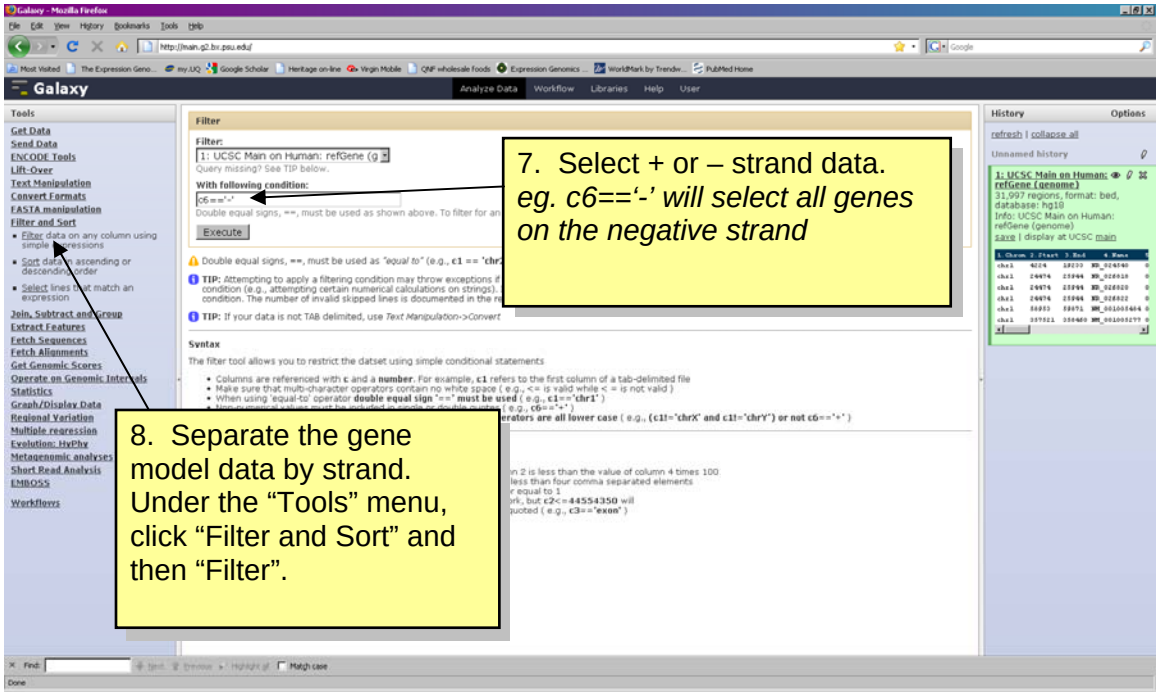
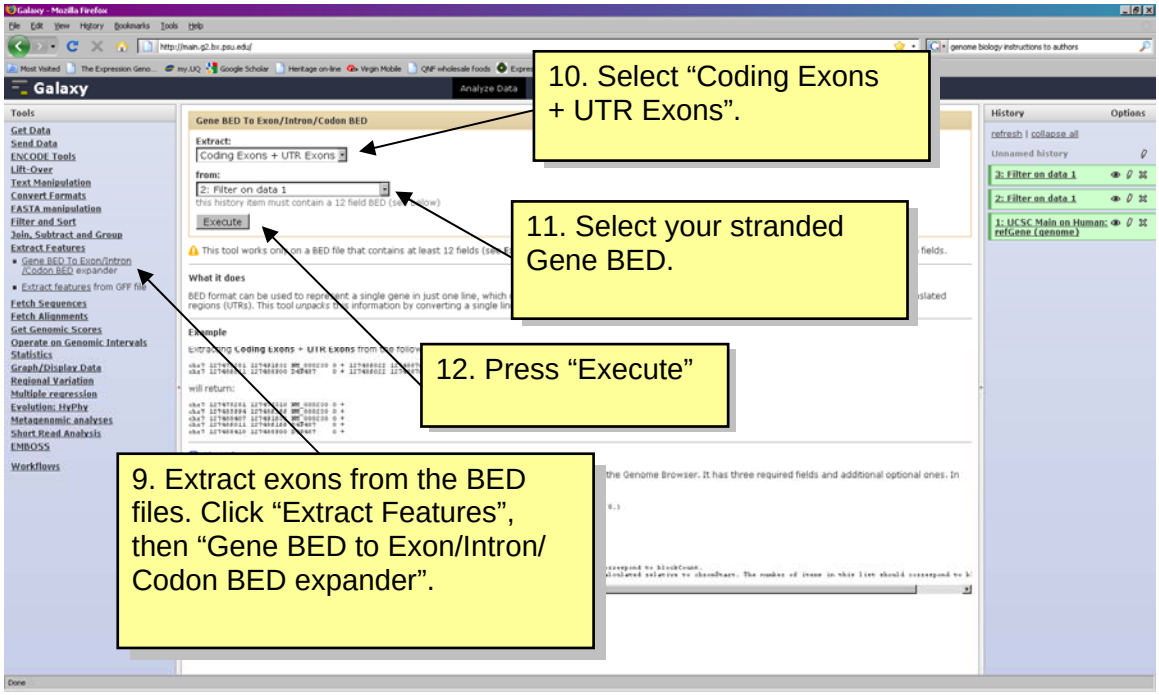
Supplementary File 1

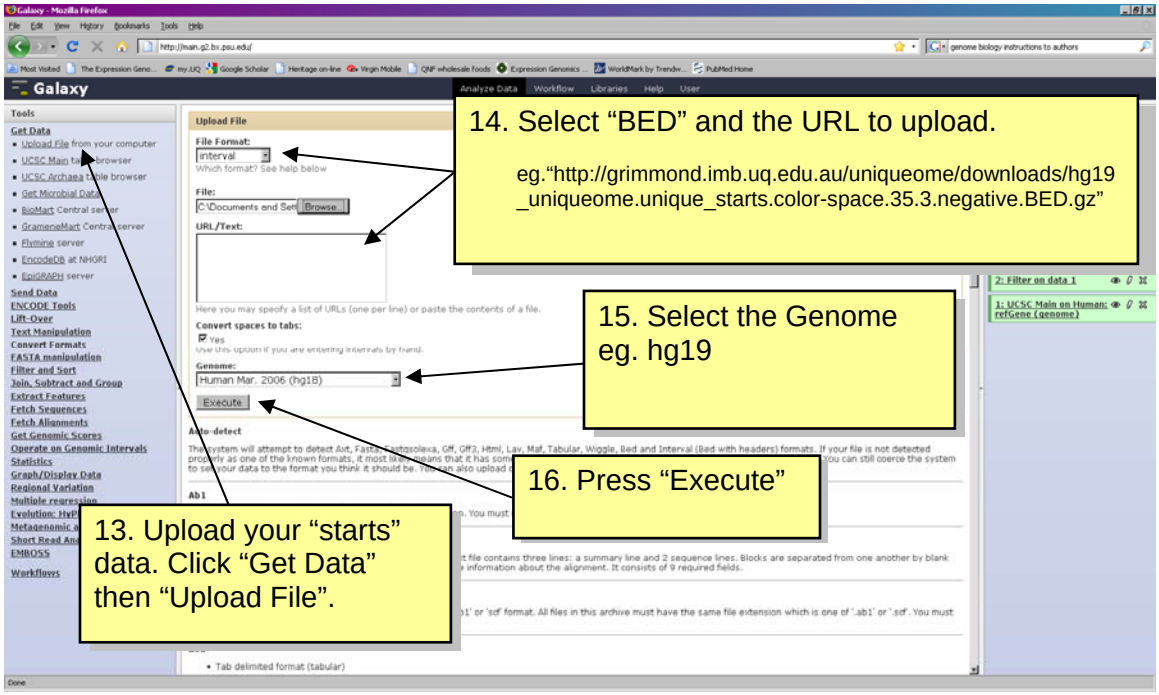
This document provides a picture based tutorial on how to use uniqueome BED files to perform genomic analyses, such as calculating the unique proportion of RefSeq genes. Galaxy can be accessed from http://main.g2.bx.psu.edu/.

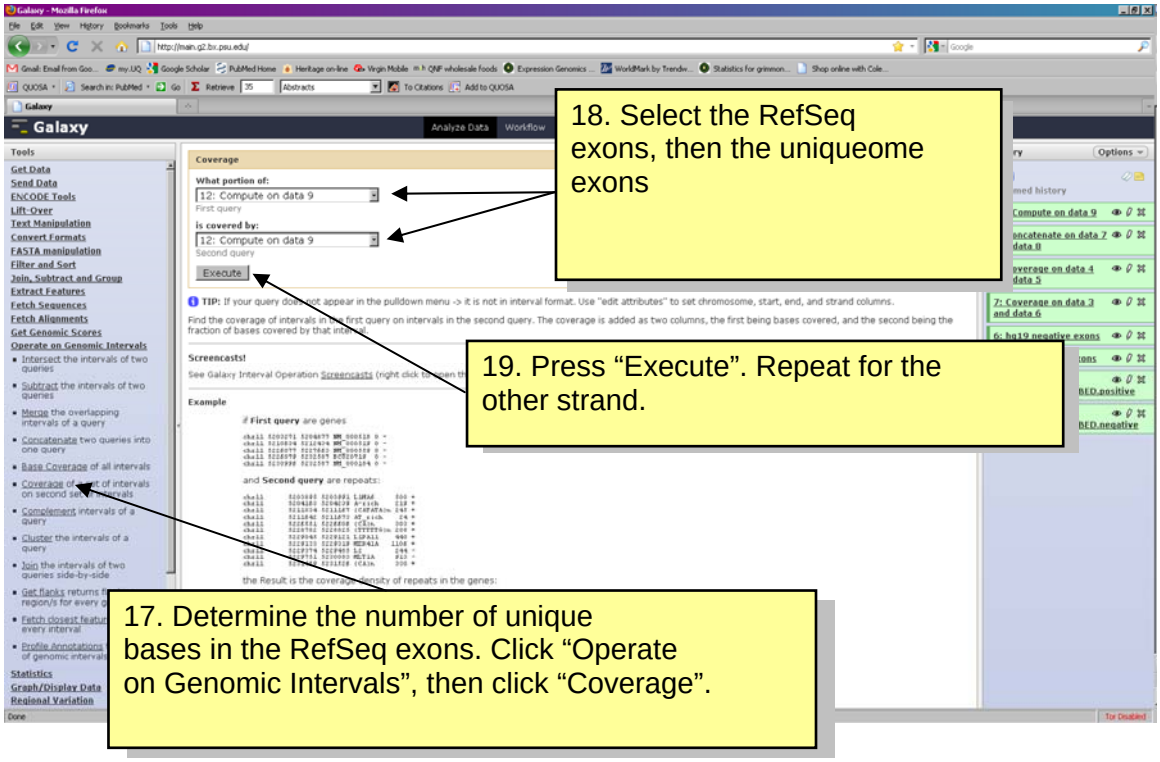


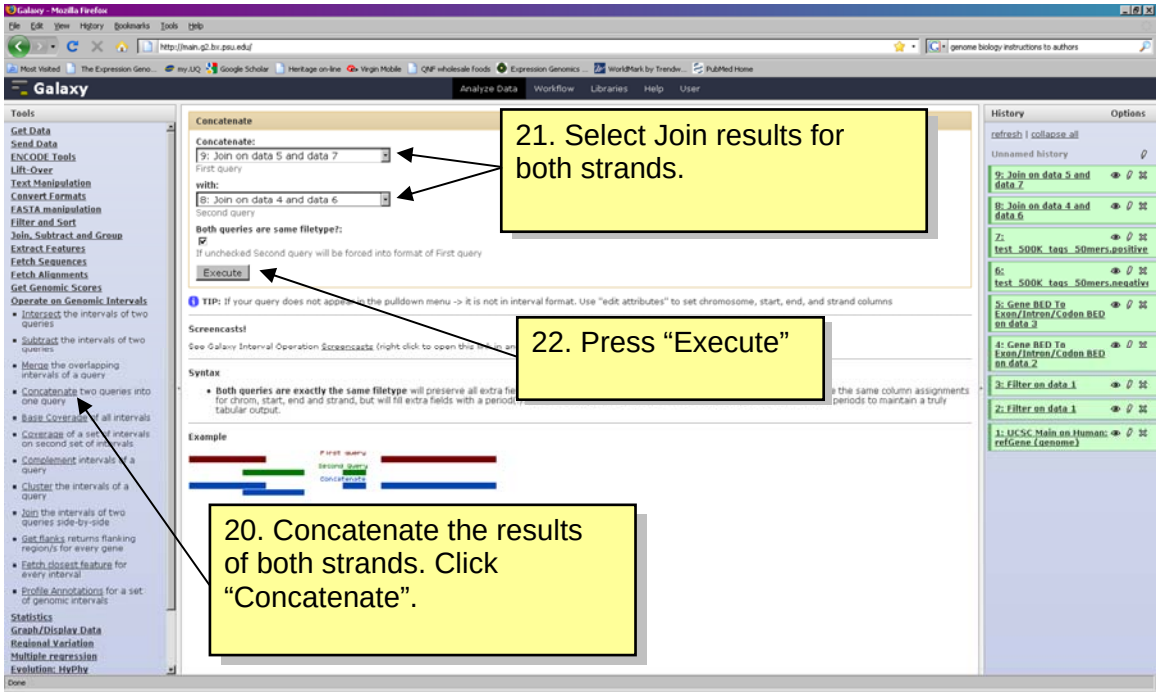


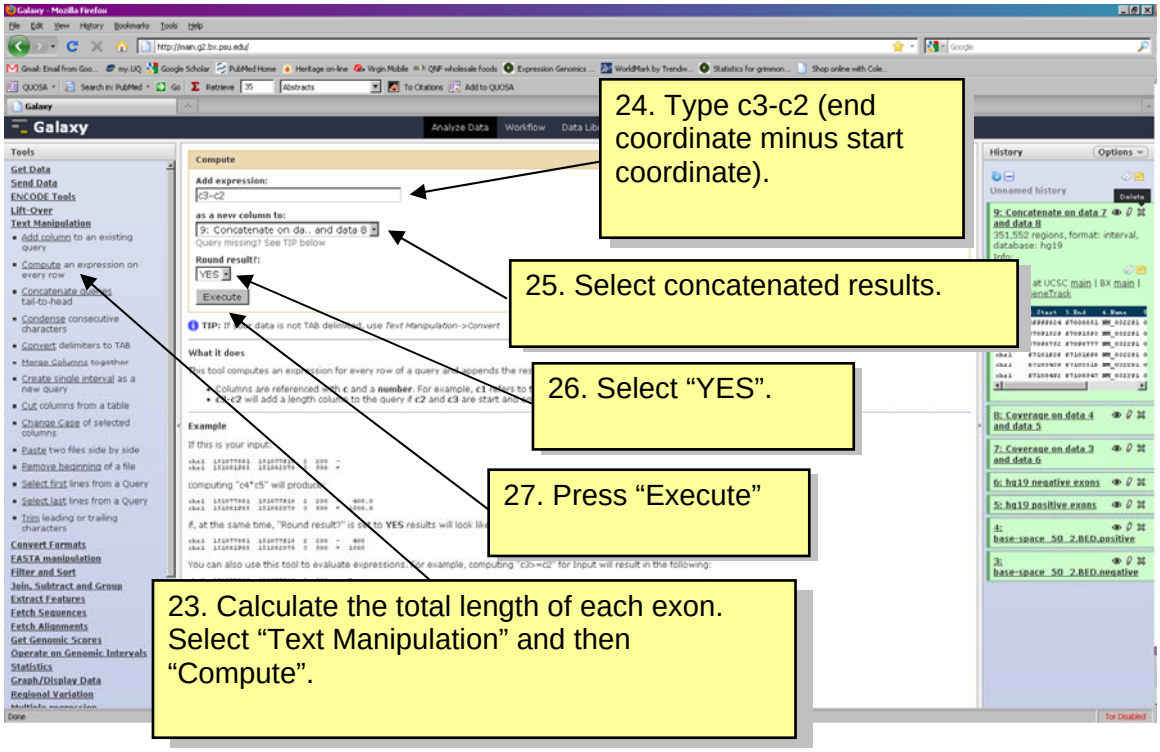

7. Select + or – strand data.
eg. c6=='-' will select all genes on the negative strand
8. Separate the gene model data by strand. Under the "Tools" menu, click "Filter and Sort" and then "Filter".

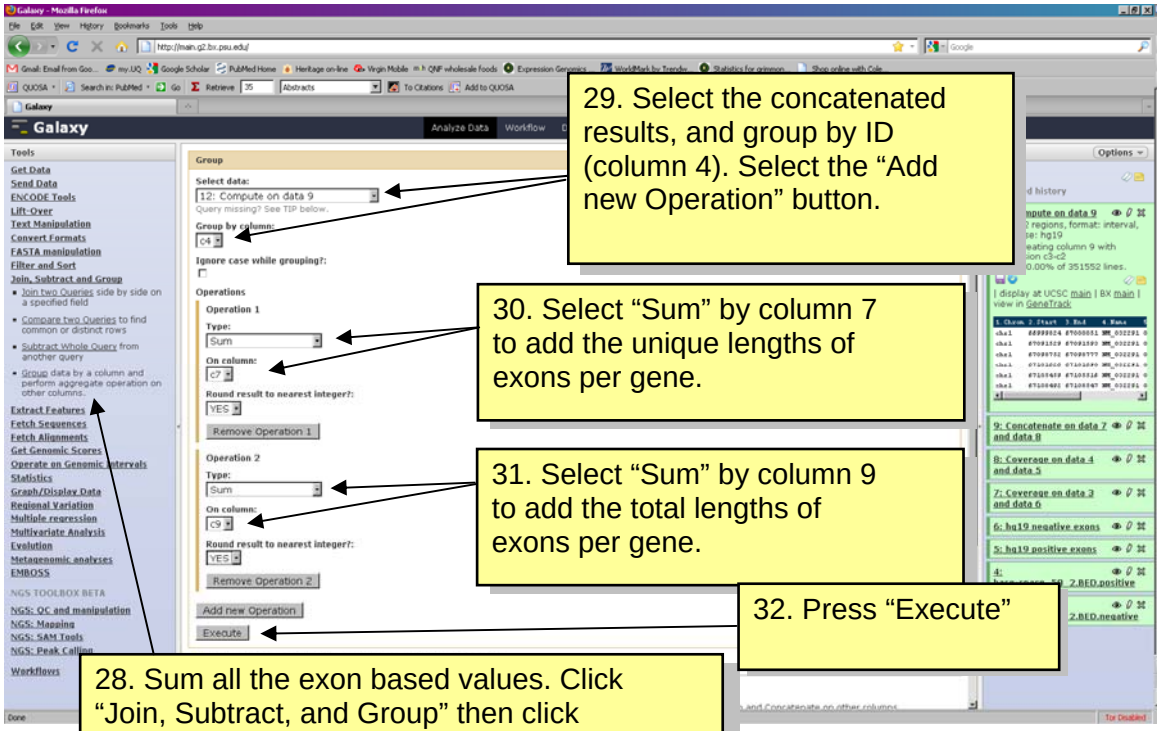

10. Select "Coding Exons + UTR Exons".
11. Select your stranded Gene BED.
12. Press "Execute"
9. Extract exons from the BED files. Click "Extract Features", then "Gene BED to Exon/Intron/ Codon BED expander".

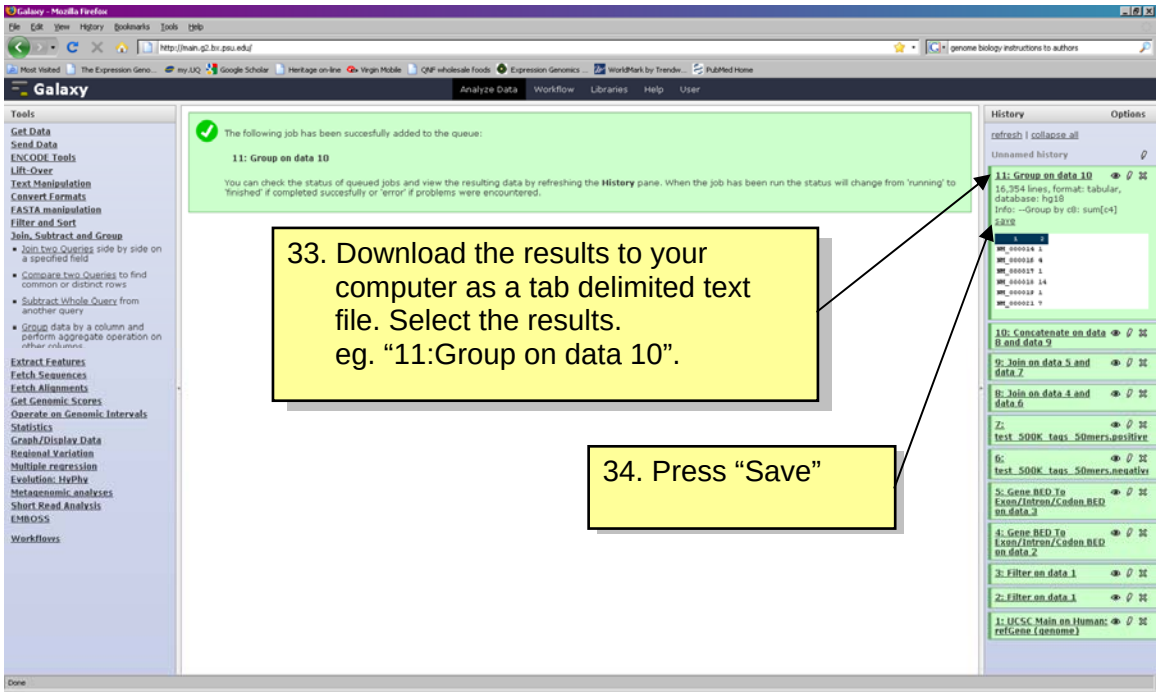

14. Select “BED” and the URL to upload.
eg.“http://grimmond.imb.uq.edu.au/uniqueome/downloads/hg19_uniqueome.unique_starts.color-space.35.3.negative.BED.gz”
15. Select the Genome eg. hg19
16. Press “Execute”
13. Upload your “starts” data. Click “Get Data” then “Upload File”.


18. Select the RefSeq exons, then the uniqueome exons
19. Press “Execute”. Repeat for the other strand.
17. Determine the number of unique bases in the RefSeq exons. Click “Operate on Genomic Intervals”, then click “Coverage”.


21. Select Join results for both strands.
22. Press “Execute”
20. Concatenate the results of both strands. Click “Concatenate”.


24. Type c3-c2 (end coordinate minus start coordinate).
25. Select concatenated results.
26. Select “YES”.
27. Press “Execute”
23. Calculate the total length of each exon. Select “Text Manipulation” and then “Compute”.


29. Select the concatenated results, and group by ID (column 4). Select the "Add new Operation" button.
30. Select "Sum" by column 7 to add the unique lengths of exons per gene.
31. Select "Sum" by column 9 to add the total lengths of exons per gene.
32. Press "Execute"
28. Sum all the exon based values. Click "Join, Subtract, and Group" then click "Group".


33. Download the results to your computer as a tab delimited text file. Select the results. eg. "11:Group on data 10".
34. Press "Save"