Working with BED Files in Galaxy and Conducting GO Enrichment Analysis

MMG1001 Assignment 4 – Group Heather (originally developed by Laura Campitelli)

Corresponding Reading

- Visel *et al.* 2009. Chip-seq accurately predicts tissue-specific activity of enhancers. Nature 457(7231): 854–858

Tools

- **UCSC Table Browser**: https://genome.ucsc.edu/cgi-bin/hgTables
- **Galaxy** (online server to execute **bedtools**): https://usegalaxy.org
- The **Gene Ontology (GO)** resource: http://geneontology.org

Additional Files

- **forebrain_peaks_p300.bed** and **limb_peaks_p300.bed** (from Visel *et al*.'s supplemental tables 2 and 4, modified to remove extra columns and headers)

Overview

The authors of the paper found that forebrain p300 peaks were particularly enriched 10kb up- or downstream of genes expressed in E11.5 forebrain tissue. We will follow a workflow to identify what genes are within this 10kb region of the p300 peaks and perform a GO enrichment analysis to see what molecular functions these genes have.

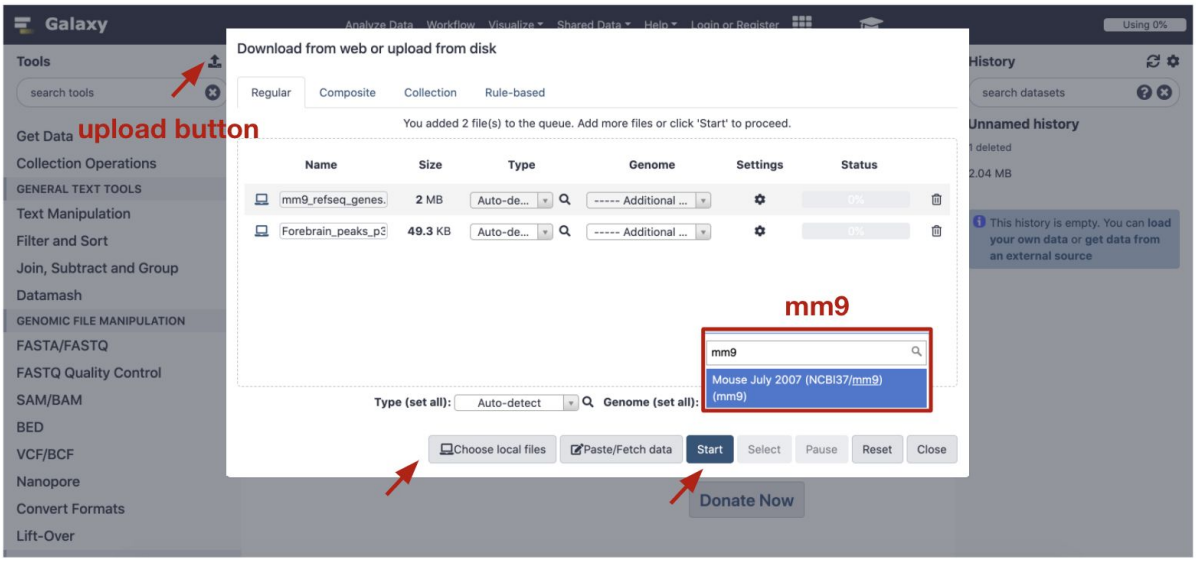
Step 1: Download BED file of mouse mm9 genes

a. Go to the **UCSC Table Browser** and select the following options to download a BED file of all the mouse genes with **RefSeq** annotations under the **mm9** reference genome, provide an informative name for the output file (such as **mm9_refseq_genes.bed**), then click *get output*:

b. On the new page, leave the default settings as they are (no custom header, one BED record per whole gene) and click *get BED*. Save the file in a folder you'll remember

Step 2: Upload files to Galaxy

- **a.** Go to the [Galaxy] main page. The left side lists the available tools and the right side will hold the files and command history. At the top left, next to *Tools*, click the upload button
- **b.** Select *Choose local files*, then add **mm9_refseq_genes.bed.gz** and **forebrain_peaks_p300.bed**
- **c.** For *Genome (set all)*, search for **mm9** and click *Start*. When the files turn green, click *Close* to exit the window
- **d.** When your files have finished uploading to Galaxy, they will turn green on the right side of the screen

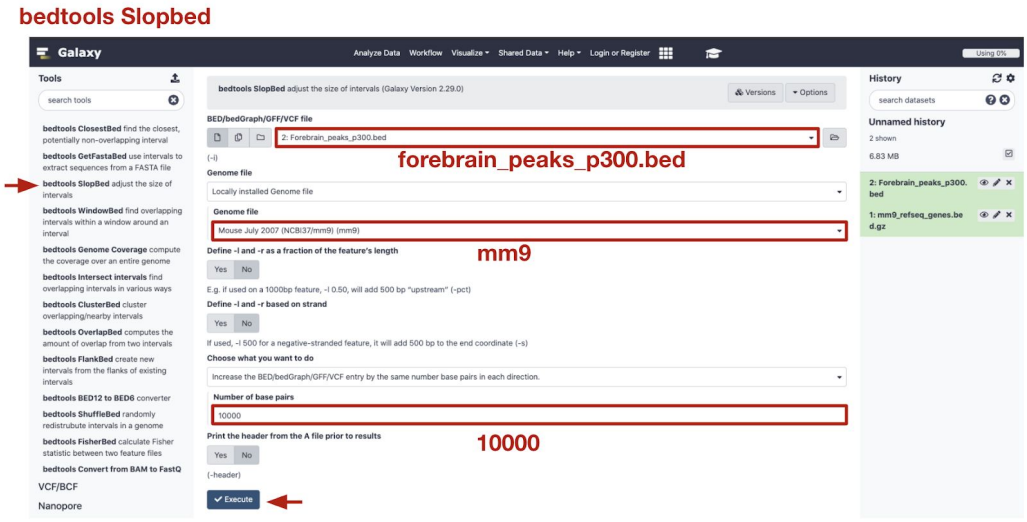


Step 3: Use bedtools slop to add 10kb flanks to p300 peaks

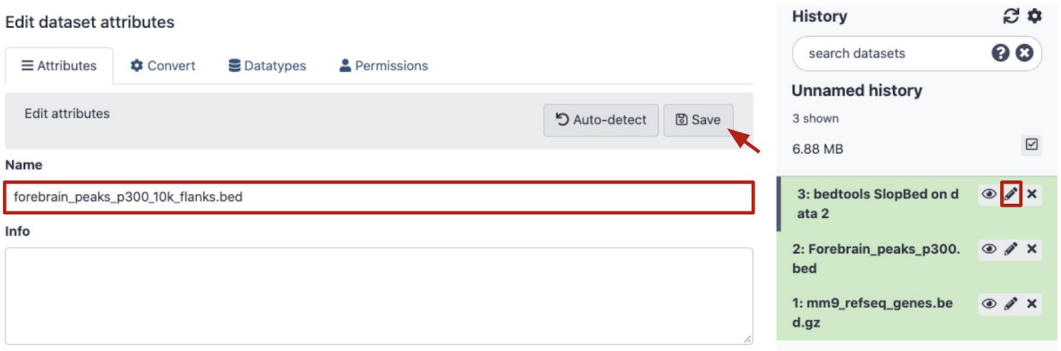
a. On the left side of Galaxy under *BED*, select **bedtools SlopBed**

b. Specify the BED file to be **forebrain_peaks_p300.bed** and select **mm9** for the *Genome file*

c. Keep all default parameters except for *Number of base pairs*, which should be changed to **10000**. Click *Execute*

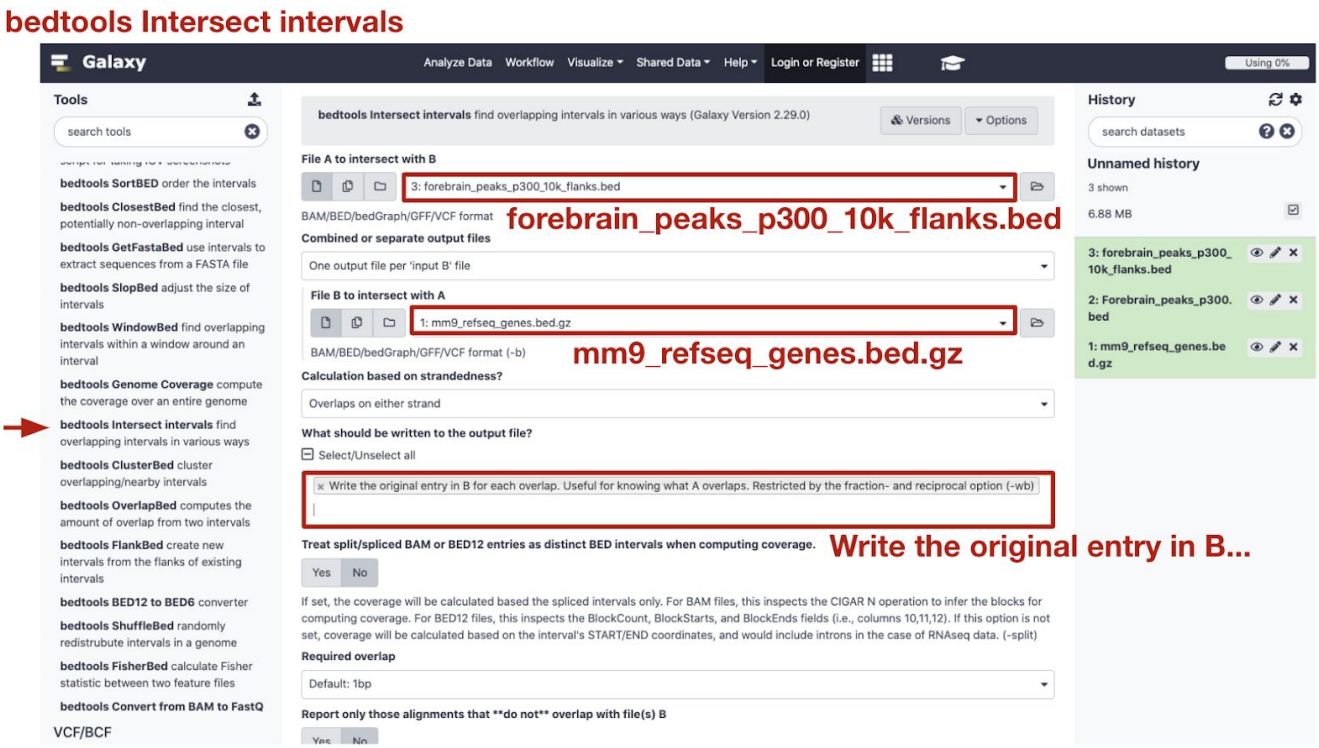


d. A new file will appear in your history on the right, named something like *bedtools SlopBed on data 2*. We can change this to a more informative name like **forebrain_peaks_p300_10k_flanks.bed** by clicking the pencil next to the new file, editing the name, and clicking *Save*. This new file contains the locations of the p300 peaks in forebrain tissue with coordinates extended by 10kb in both directions



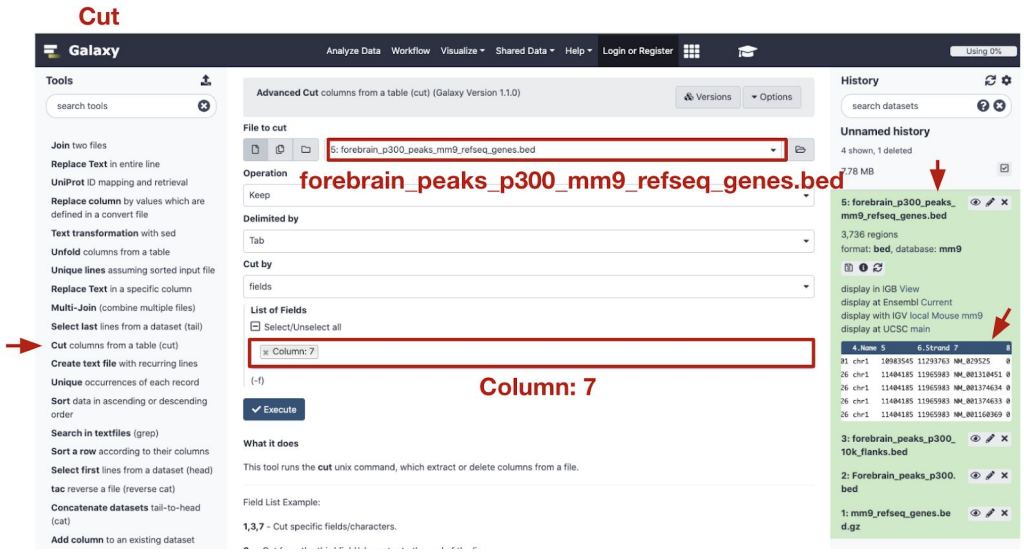
Step 4: Use bedtools intersect to identity mouse genes within 10kb of p300 peaks

a. On the left side of Galaxy under *BED*, select **bedtools Intersect intervals**
b. Specify *File A* to be the newly created **forebrain_peaks_p300_10k_flanks.bed**
c. Specify *File B* to be **mm9_refseq_genes.bed.gz**
d. For *What should be written to the output file*, select **Write the original entry in B for each overlap…**
e. Keep the remaining default parameters and click *Execute*. Rename the new file **forebrain_peaks_p300_mm9_refseq_genes.bed**. This new file filtered down the original bed file of mouse genes to only those genes that were found within 10kb of the forebrain p300 peaks

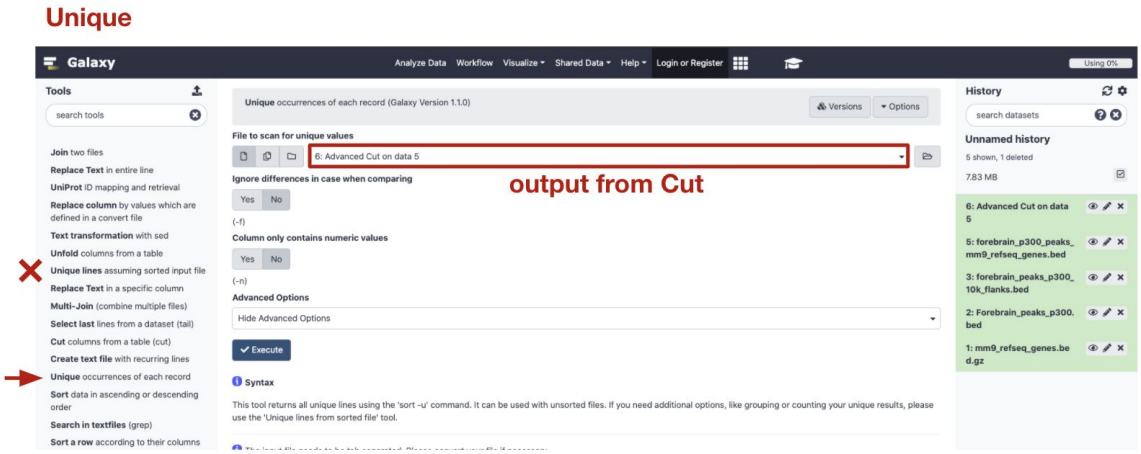


Step 5: Obtain the list of RefSeq genes found within 10kb of p300 forebrain peaks

- **a.** On the left side of Galaxy under *Text Manipulation*, select *Cut*
- **b.** Specify *File to cut* to be **forebrain_peaks_p300_mm9_refseq_genes.bed**
- **c.** When you click on **forebrain_peaks_p300_mm9_refseq_genes.bed** on the right side, you can see a snippet of the data it contains. If you scroll to the right you can see that the RefSeq names are in column 7. Back in the middle of the page, under *List of Fields*, select **Column: 7**
- **d.** Keep the remaining default parameters and click *Execute*. The new file will be just the list of RefSeq genes, but it might contain duplicate entries



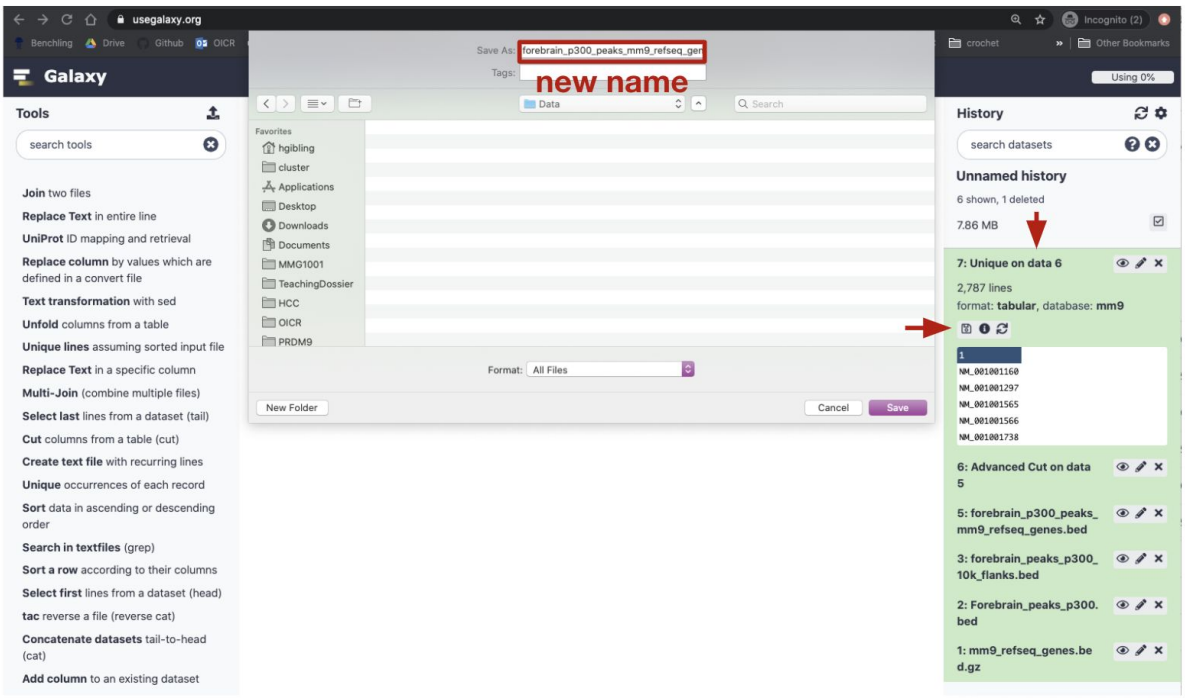
- **e.** On the left side of Galaxy under *Text Manipulation*, select *Unique* (**important**: make sure you do **not** select *Unique lines*, which should also work in theory, but was giving weird results when testing)
- **f.** Specify *File to scan for unique values* to be the output of the last command (something like *Advanced Cut on data 4*)
- **g.** Keep the remaining default parameters and click *Execute*. The new file will be the sorted list of unique RefSeq genes (no duplicate entries)

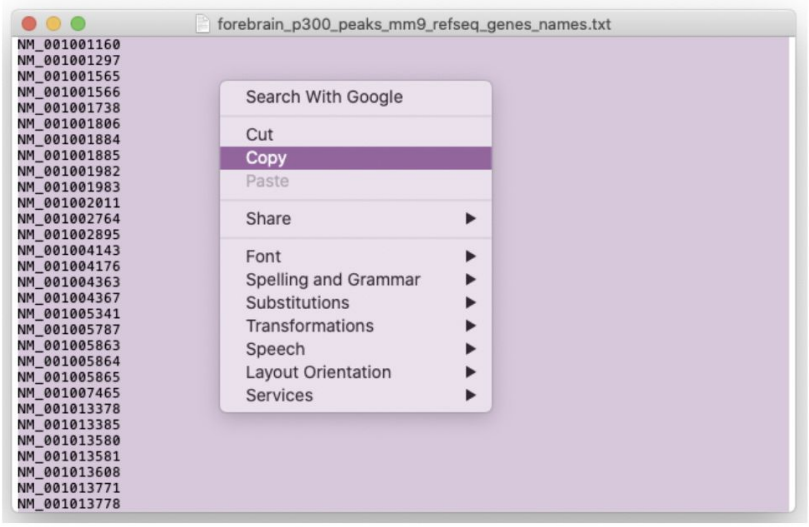


Step 6: Download files from Galaxy

a. Download the new file by clicking on the name on the right side of the page and clicking on the floppy disc icon. Rename it **forebrain_peaks_p300_mm9_refseq_genes_names.txt** and save it in a folder you'll remember

b. Open the file (in a plain text editor), do *control-a* or *command-a* to select all of the text, and then copy the text

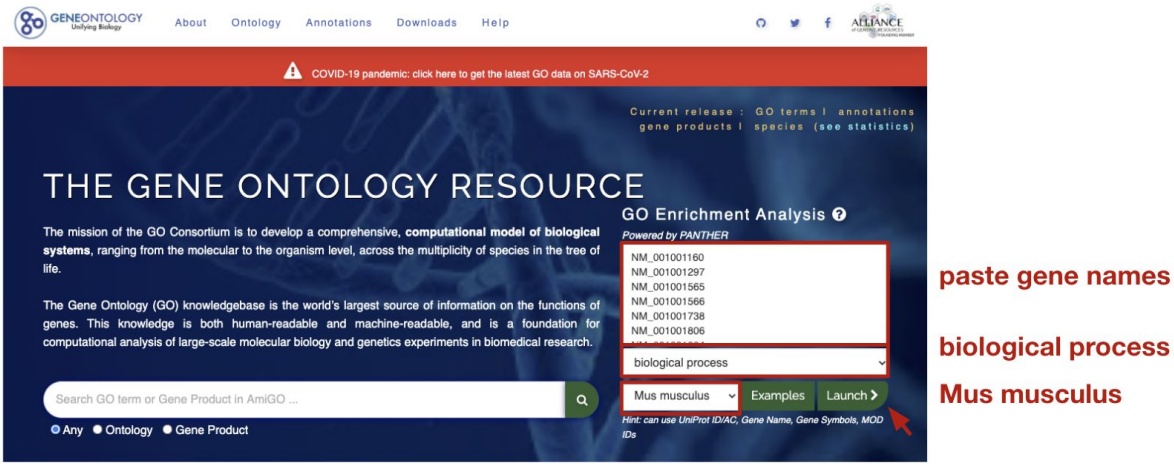




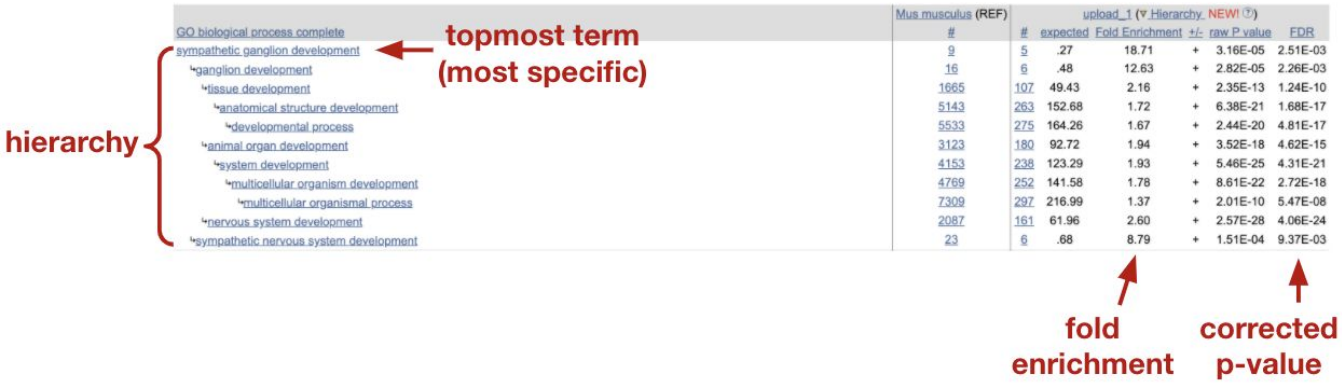
Step 7: Perform GO enrichment analysis for the genes within 10kb of p300 forebrain peaks

a. Go to the **Gene Ontology** page and paste in the list of RefSeq genes into the box on the right

b. Select *biological process* and *Mus musculus*, then click *Launch*



c. **Pather** software performs an enrichment analysis of the GO terms associated with each of the input genes, using *Fisher's Exact test* and *FDR correction* as defaults. The default display groups the GO terms by hierarchy, in decreasing order of fold enrichment (for the topmost term in the hierarchy)



GO biological process complete	Mus musculus (REF) #	upload_1 (Hierarchy NEW!) #	expected	Fold Enrichment	+/-	raw P value	FDR
sympathetic ganglion development	9	5	.27	18.71	+	3.16E-05	2.51E-03
ganglion development	16	6	.48	12.63	+	2.82E-05	2.26E-03
tissue development	1665	107	49.43	2.16	+	2.35E-13	1.24E-10
anatomical structure development	5143	263	152.68	1.72	+	6.38E-21	1.68E-17
developmental process	5533	275	164.26	1.67	+	2.44E-20	4.81E-17
animal organ development	3123	180	92.72	1.94	+	3.52E-18	4.62E-15
system development	4153	238	123.29	1.93	+	5.46E-25	4.31E-21
multicellular organism development	4769	252	141.58	1.78	+	8.61E-22	2.72E-18
multicellular organismal process	7309	297	216.99	1.37	+	2.01E-10	5.47E-08
nervous system development	2087	161	61.96	2.60	+	2.57E-28	4.06E-24
sympathetic nervous system development	23	6	.68	8.79	+	1.51E-04	9.37E-03

Questions

1. Which GO terms related to forebrain development are enriched, and what is the fold enrichment and corrected p-value? (topmost (most specific) term is fine)
2. Why are there enriched GO terms not related to forebrain development?
3. Why is looking at GO enrichment useful (compared to looking at lists of enriched genes)?

If you have time, repeat steps 2-7 with the limb p300 peaks. It might help to first delete all your Galaxy files except for mm9_refseq_genes.bed.gz (or make sure they are clearly named 'forebrain').

Which GO terms related to limb development are enriched? Are any GO terms enriched in both limb and forebrain?