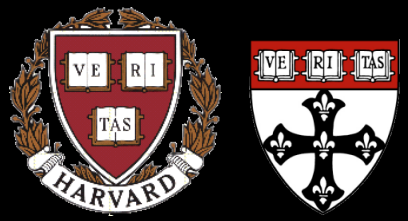
Amplicon functional profiling with PICRUSt

Curtis Huttenhower

01-19-15



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Department of Biostatistics

U. Oregon

The two big questions…

Who is there?
(taxonomic profiling)

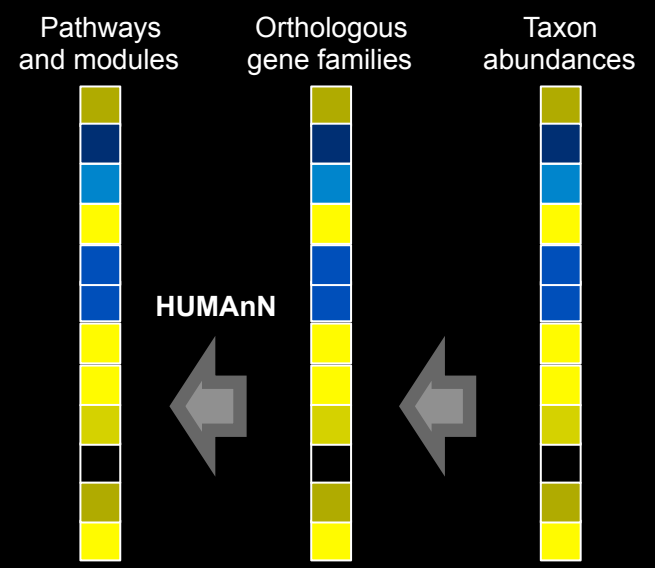
What are they doing?
(functional profiling)

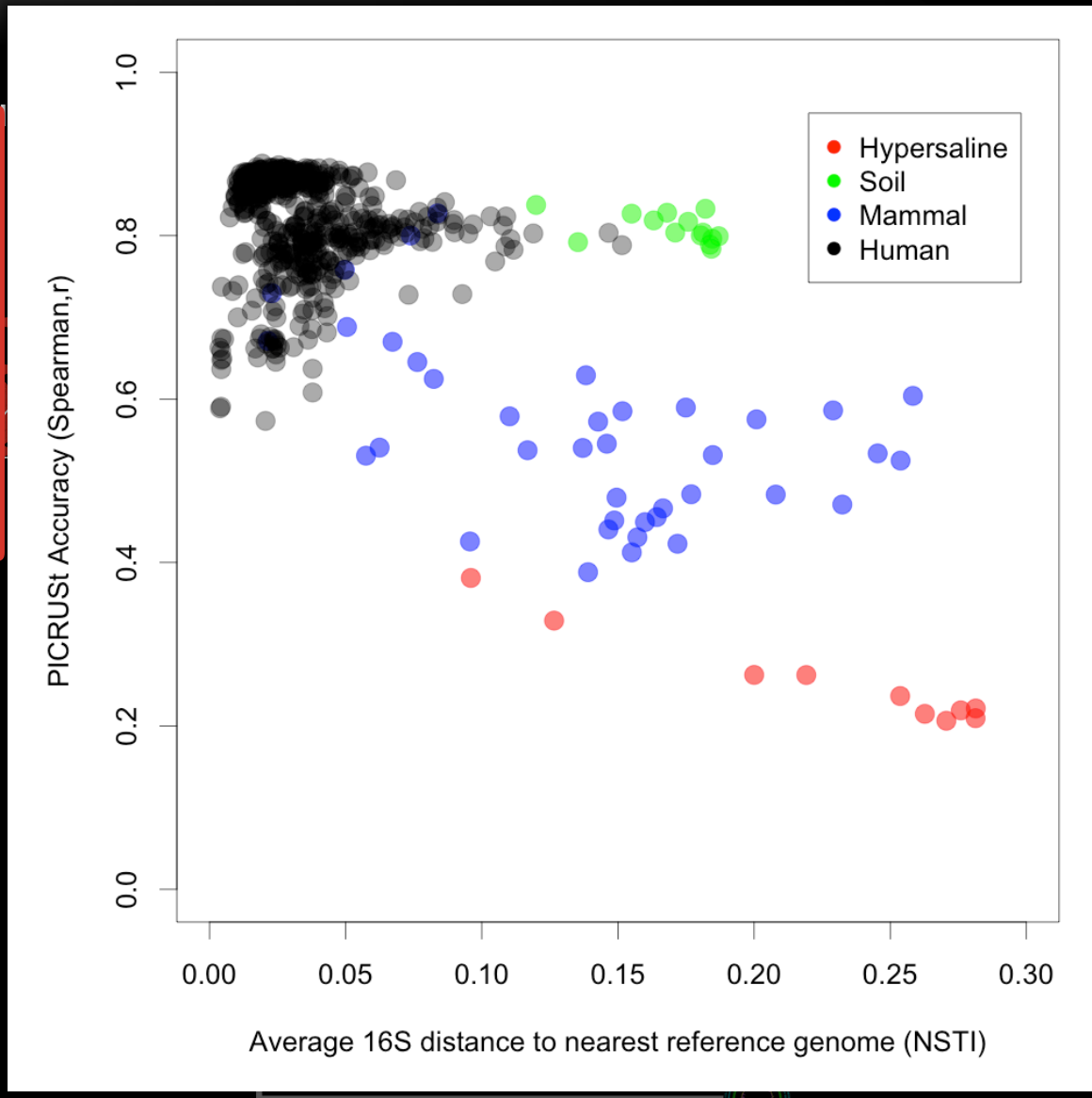
In marker gene data

PICRUSt: Inferring community metagenomic potential from marker gene sequencing

With Rob Knight, Rob Beiko

If function is so important, what about the thousands of 16S-based microbial community taxonomic profiles?





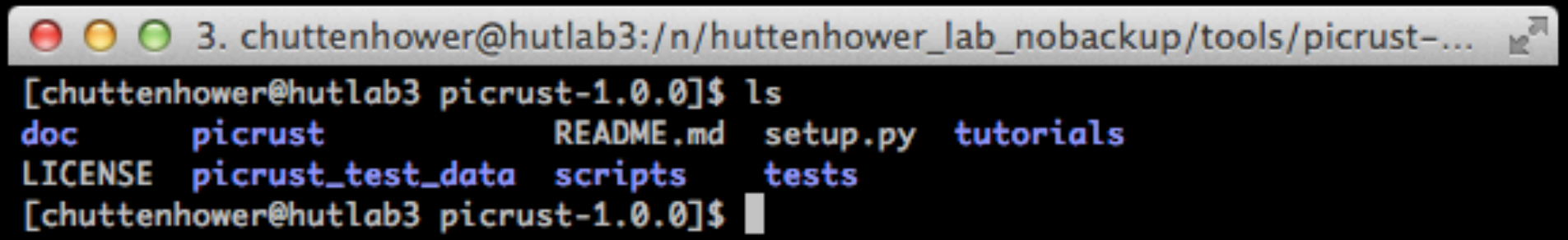
Relative abundance

Setup notes reminder

- Slides with green titles or text include instructions not needed today, but useful for your own analyses
- Keep an eye out for red warnings of particular importance
- Command lines and program/file names appear in a `monospaced font`.
- Commands you should specifically copy/paste are in **`monospaced bold blue`**.

Installing PICRUSt

- http://picrust.github.io/picrust/install.html
- Requires
  - Python (>=2.7, easy)
  - PyCogent (http://pycogent.org)
  - BIOM (http://biom-format.org)
- And PICRUSt!



Installing PICRUSt

- http://picrust.github.io/picrust/install.html

Installing PICRUSt

Note

Most users will not need to install PICRUSt, but can instead use the online Galaxy version.

PICRUSt is written in python, and has been tested on Mac OS X and Linux systems. To install PICRUSt, first install all of the mandatory requirements, following the instructions on their respective websites. You should then download PICRUSt. You have the choice of downloading either the release version (recommended for most users) or the development version (recommended for users who need access to the latest features, and are willing to tolerate some instability in the code). Next, you will download the large precalculated PICRUSt files and place them in your picrust/data directory. Finally, you'll install PICRUSt. Each of these steps are detailed below.

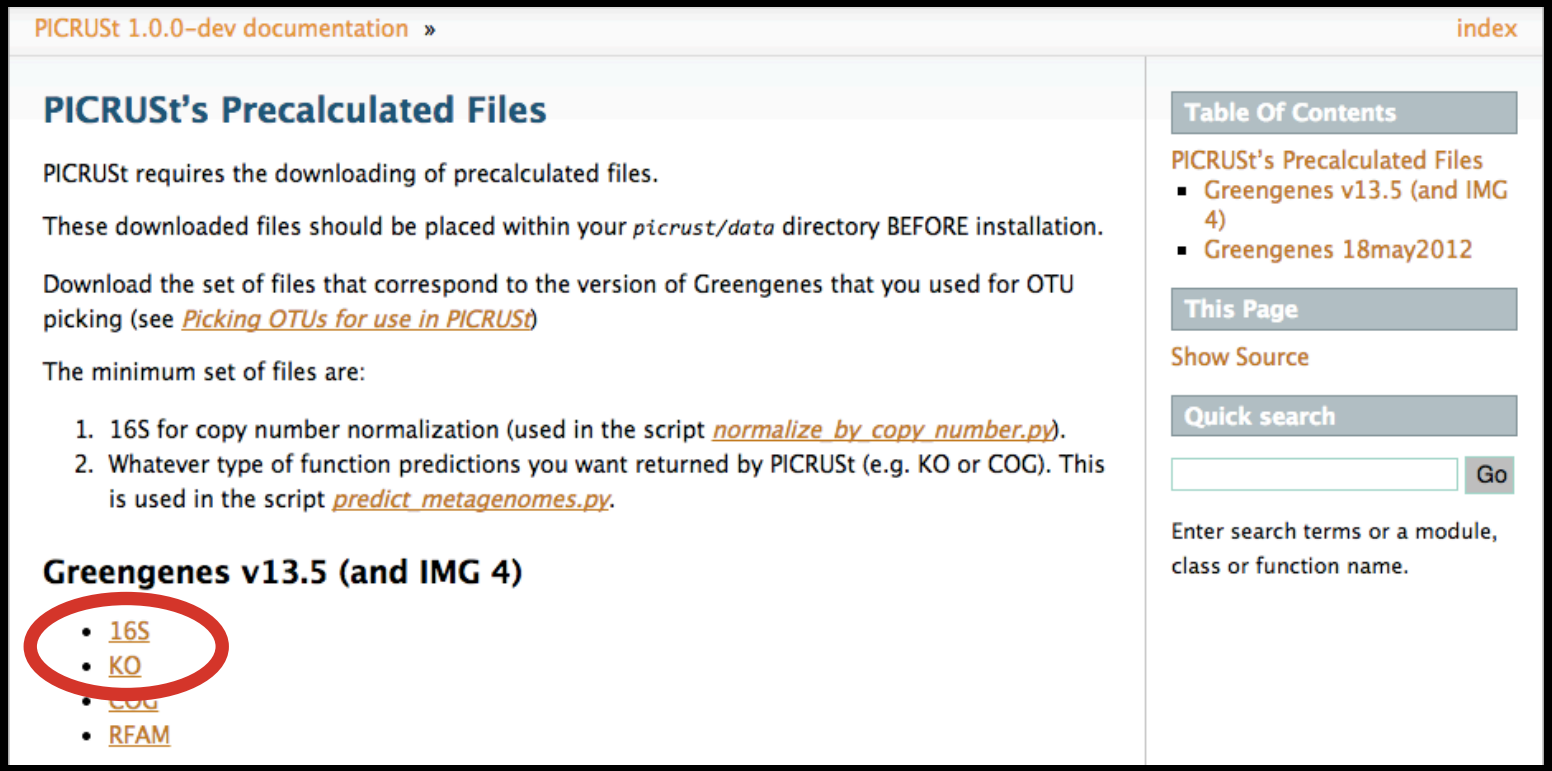
Step 1. Install Requirements

Follow the install instructions found on the website of each of the dependencies below to install PICRUSt's dependencies.

Installing PICRUSt data

- http://picrust.github.io/picrust/picrust_precalculated_files.html
- Need to download the precomputed data used by PICRUSt separately
  - It's big! Saves you the trouble in the software itself

These files must be placed in your `picrust-1.0.0/picrust/data` directory before installing.



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PICRUSt's Precalculated Files

PICRUSt requires the downloading of precalculated files.

These downloaded files should be placed within your *picrust/data* directory BEFORE installation.

Download the set of files that correspond to the version of Greengenes that you used for OTU picking (see *Picking OTUs for use in PICRUSt*)

The minimum set of files are:

1. 16S for copy number normalization (used in the script *normalize_by_copy_number.py*).
2. Whatever type of function predictions you want returned by PICRUSt (e.g. KO or COG). This is used in the script *predict_metagenomes.py*.

Greengenes v13.5 (and IMG 4)

- 16S
- KO
- COG
- RFAM

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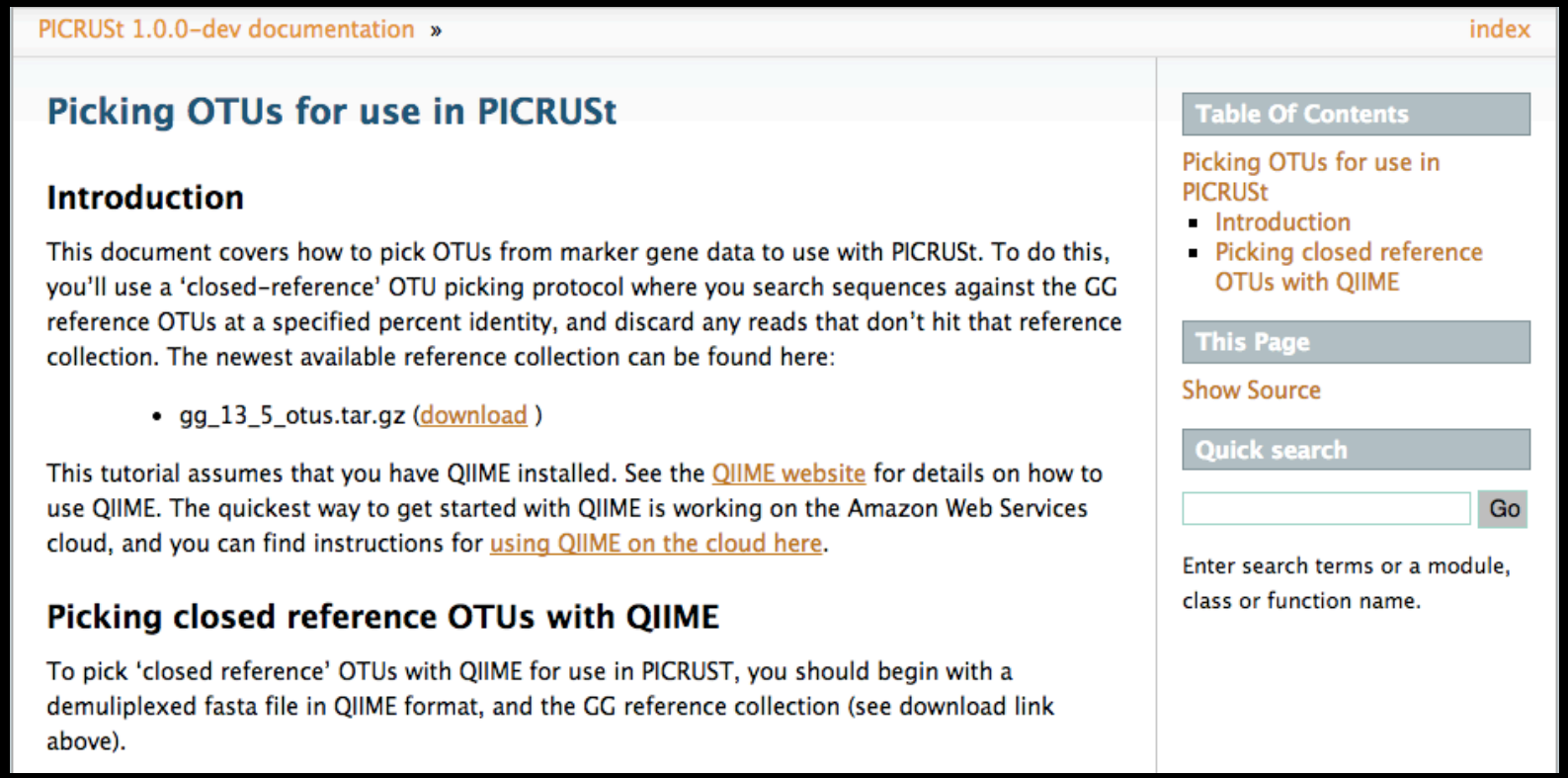
Picking PICRUSt-compatible OTUs

- http://picrust.github.io/picrust/tutorials/otu_picking.html
- PICRUSt uses precomputed ancestral state reconstructions
  - OTUs in your data must match those used during precalculation
  - This means Greengenes, either 18may2012 or 13.5



Picking PICRUSt-compatible OTUs

- This means that you must use completely closed-reference OTU picking in QIIME

```
echo "pick_otus:enable_rev_strand_match True"  >> $PWD/otu_picking_params_97.txt
echo "pick_otus:similarity 0.97" >> $PWD/otu_picking_params_97.txt
pick_closed_reference_otus.py -i $PWD/seqs.fna -o $PWD/ucrC97/ -p $PWD/otu_picking_params
```

- Produces an OTU table in which all features are Greengenes IDs:

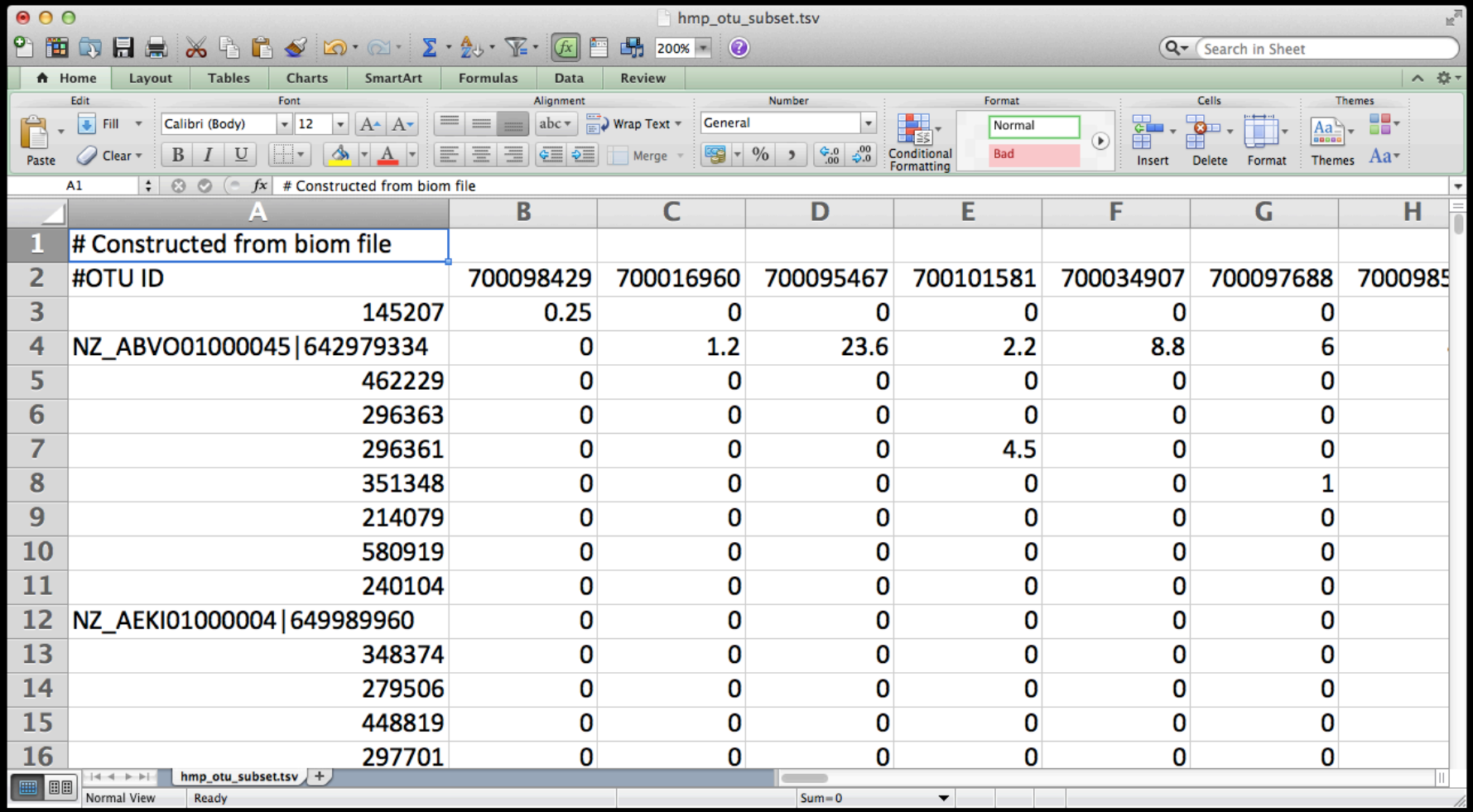
Picking PICRUSt-compatible OTUs

3. chuttenhower@hutlab3:~/stamps (ssh)

```
# Constructed from biom file
#OTU ID 700098429   700016960   700095467   700101581   700034907   700097688
145207  0.25    0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
NZ_ABVO01000045|642979334   0.0 1.2 23.6    2.2 8.8 6.0 4.4 0.2 0.2 119.4   7.0
462229  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
296363  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
296361  0.0 0.0 0.0 4.5 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
351348  0.0 0.0 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 1.0 0.0 0.0 1.0 0.0 0.0 1.0
214079  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 89.0    0.0 0.0 0.0 16.4    0.0
580919  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
240104  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
NZ_AEKI01000004|649989960   0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
348374  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
279506  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
448819  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
297701  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
386632  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
376175  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
NC_011601|643348548 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
NZ_ACIJ02000018|645951840   0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
223033  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
189116  14.3333333333   0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
545964  0.0 0.0 0.0 0.0 0.666666666667  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
128297  0.666666666667  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
```

hmp_otu_subset.tsv

Picking PICRUSt-compatible OTUs



	A	B	C	D	E	F	G	H
1	# Constructed from biom file							
2	#OTU ID	700098429	700016960	700095467	700101581	700034907	700097688	7000985
3	145207	0.25	0	0	0	0	0	
4	NZ_ABVO01000045|642979334	0	1.2	23.6	2.2	8.8	6	
5	462229	0	0	0	0	0	0	
6	296363	0	0	0	0	0	0	
7	296361	0	0	0	4.5	0	0	
8	351348	0	0	0	0	0	1	
9	214079	0	0	0	0	0	0	
10	580919	0	0	0	0	0	0	
11	240104	0	0	0	0	0	0	
12	NZ_AEKI01000004|649989960	0	0	0	0	0	0	
13	348374	0	0	0	0	0	0	
14	279506	0	0	0	0	0	0	
15	448819	0	0	0	0	0	0	
16	297701	0	0	0	0	0	0	

Munging BIOMs

- Great format, hard to read!
  - Easy to convert to/from TSV

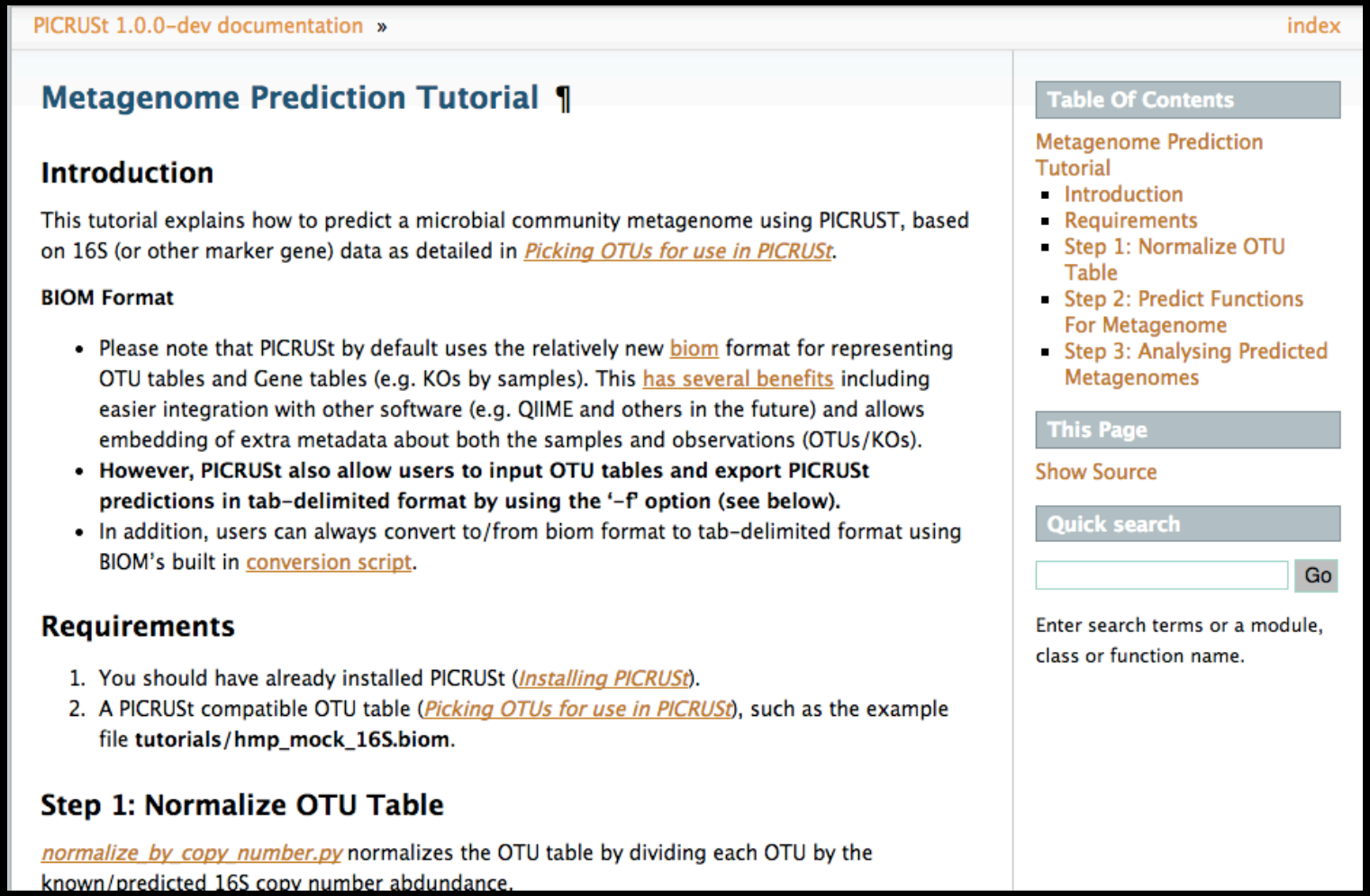
```
convert_biom.py -i hmp_otu_subset.tsv
  -o hmp_otu_subset.biom
  --biom_table_type="otu table"
```

3. chuttenhower@hutlab3:~/stamps (ssh)


```
{"id": "None","format": "Biological Observation Matrix 1.0.0","format_url": "htt
p://biom-format.org","type": "OTU table","generated_by": "BIOM-Format 1.1.2","da
te": "2014-08-15T18:17:31.215210","matrix_type": "sparse","matrix_element_type":
 "float","shape": [3034, 100],"data": [[0,0,0.25],[1,1,1.2],[1,2,23.600000000000
001],[1,3,2.2000000000000002],[1,4,8.8000000000000007],[1,5,6.0],[1,6,4.40000000
00000004],[1,7,0.20000000000000001],[1,8,0.20000000000000001],[1,9,119.400000000
00001],[1,10,7.0],[1,11,26.199999999999999],[1,12,3.3999999999999999],[1,13,0.40
000000000000002],[1,14,0.40000000000000002],[1,15,1.8],[1,16,29.800000000000001]
,[1,17,55.799999999999997],[1,18,0.20000000000000001],[2,19,0.25],[2,20,0.25],[2
,21,1.0],[2,22,0.25],[2,23,0.5],[2,24,0.25],[2,25,0.75],[2,26,1.75],[2,27,0.75],
[2,28,0.25],[2,29,0.25],[2,30,0.75],[2,31,0.5],[3,32,1.0],[4,3,4.5],[4,18,1.5],[
4,33,8.5],[4,34,3.5],[4,35,272.0],[4,36,842.5],[5,5,1.0],[5,11,1.0],[5,14,1.0],[
5,17,1.0],[5,18,3.0],[5,35,1.0],[5,36,1.0],[5,37,1.0],[5,38,1.0],[5,39,1.0],[5,4
0,1.0],[5,41,1.0],[5,42,1.0],[5,43,1.0],[5,44,1.0],[5,45,1.0],[5,46,4.0],[5,47,1
.0],[5,48,1.0],[5,49,2.0],[5,50,2.0],[5,51,4.0],[5,52,5.0],[5,53,4.0],[5,54,1.0]
```

Everything I ever needed to know about PICRUSt I learned from this web site

- http://picrust.github.io/picrust/tutorials/metagenome_prediction.html



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Metagenome Prediction Tutorial ¶

Introduction

This tutorial explains how to predict a microbial community metagenome using PICRUST, based on 16S (or other marker gene) data as detailed in *Picking OTUs for use in PICRUSt*.

BIOM Format

- Please note that PICRUSt by default uses the relatively new biom format for representing OTU tables and Gene tables (e.g. KOs by samples). This has several benefits including easier integration with other software (e.g. QIIME and others in the future) and allows embedding of extra metadata about both the samples and observations (OTUs/KOs).
- **However, PICRUSt also allow users to input OTU tables and export PICRUSt predictions in tab-delimited format by using the '-f' option (see below).**
- In addition, users can always convert to/from biom format to tab-delimited format using BIOM's built in conversion script.

Requirements

1. You should have already installed PICRUSt (*Installing PICRUSt*).
2. A PICRUSt compatible OTU table (*Picking OTUs for use in PICRUSt*), such as the example file **tutorials/hmp_mock_16S.biom**.

Step 1: Normalize OTU Table

normalize_by_copy_number.py normalizes the OTU table by dividing each OTU by the known/predicted 16S copy number abundance.

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Step 1: Normalize OTUs by 16S copy number

- We can get better predictions by dividing “raw” OTU counts by their expected 16S copy number

PLoS Comput Biol. 2012;8(10):e1002743. doi: 10.1371/journal.pcbi.1002743. Epub 2012 Oct 25.

Incorporating 16S gene copy number information improves estimates of microbial diversity and abundance.

Kembel SW[1], Wu M, Eisen JA, Green JL.

Microbiome. 2014 Apr 7;2:11. doi: 10.1186/2049-2618-2-11. eCollection 2014.

CopyRighter: a rapid tool for improving the accuracy of microbial community profiles through lineage-specific gene copy number correction.

Angly FE[1], Dennis PG[2], Skarshewski A[1], Vanwonterghem I[3], Hugenholtz P[1], Tyson GW[3].

```
export PYTHONPATH=`pwd`/picrust-1.0.0
./picrust-1.0.0/scripts/normalize_by_copy_number.py
  -i hmp_otu_subset.biom
  -o hmp_otu_subset_normalized.biom
  -g 18may2012
```

Step 1: Normalize OTUs by 16S copy number

```
convert_biom.py -i hmp_otu_subset_normalized.biom
  -o hmp_otu_subset_normalized.tsv -b
```

3. chuttenhower@hutlab3:~/stamps (ssh)

```
# Constructed from biom file
#OTU ID 700098429   700016960   700095467   700101581   700034907   700097688
145207  0.0625  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
NZ_ABVO01000045|642979334   0.0 0.24    4.72    0.44    1.76    1.2 0.88    0.04
462229  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
296363  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
296361  0.0 0.0 0.0 2.25    0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
351348  0.0 0.0 0.0 0.0 0.0 1.0 0.0 0.0 0.0 0.0 0.0 1.0 0.0 0.0 1.0 0.0 0.0 1.0
214079  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 17.8    0.0 0.0 0.0 3.28    0.0
580919  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
240104  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
NZ_AEKI01000004|649989960   0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
348374  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
279506  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
448819  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
297701  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
386632  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
376175  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
NC_011601|643348548 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
NZ_ACIJ02000018|645951840   0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
223033  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
189116  4.77777777777   0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
545964  0.0 0.0 0.0 0.0 0.222222222222  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
128297  0.222222222222  0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
hmp_otu_subset_normalized.tsv
```

Step 2: Predict metagenome contents

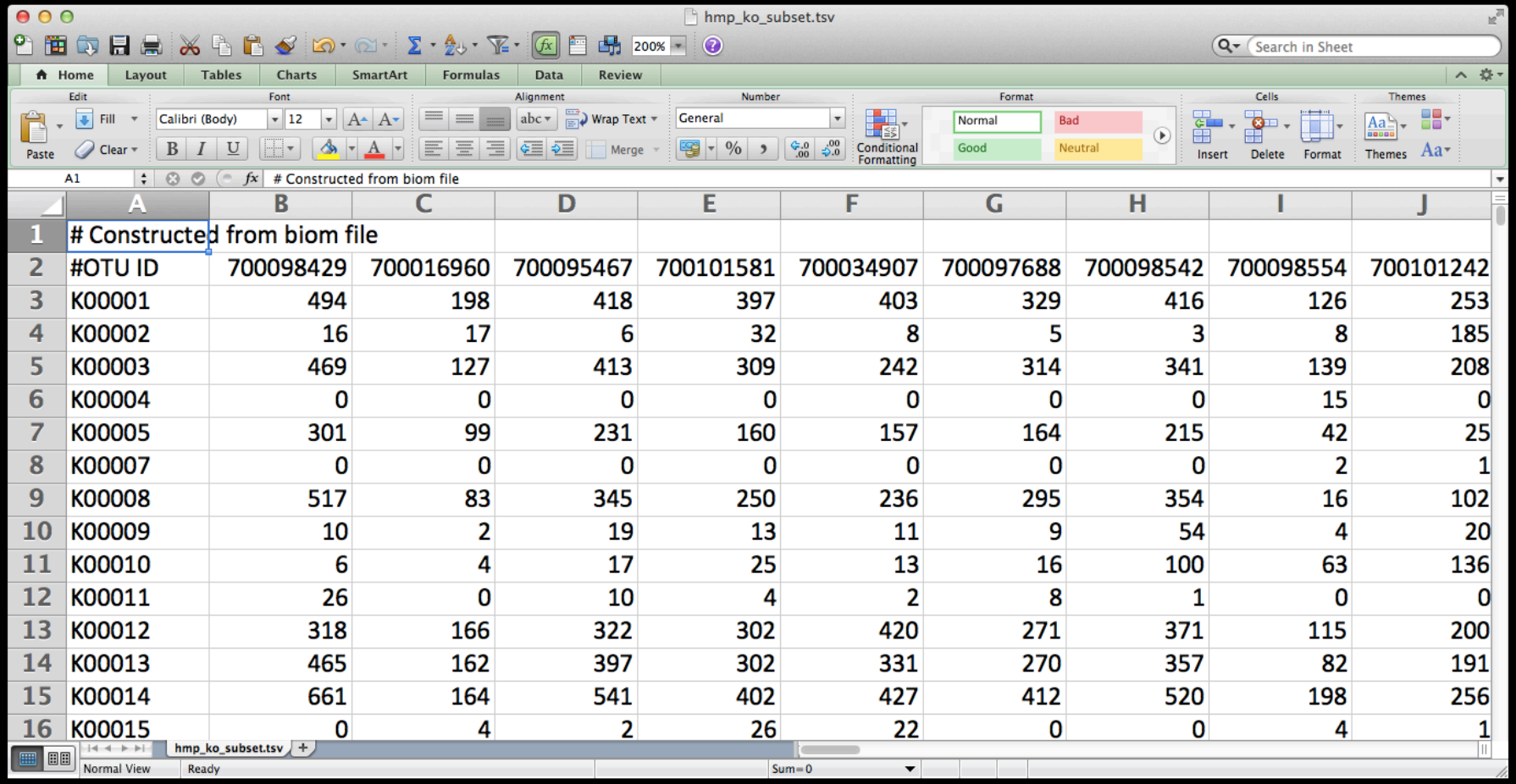
- Given a normalized OTU table, PICRUSt uses gene copy numbers associated with each Greengenes tree tip to multiply and infer community gene copy numbers

```
./picrust-1.0.0/scripts/predict_metagenomes.py
  -i hmp_otu_subset_normalized.biom
  -o hmp_ko_subset.tsv -f -g 18may2012
```

3. chuttenhower@hutlab3:~/stamps (ssh)

```
# Constructed from biom file
#OTU ID 700098429   700016960   700095467   700101581   700034907   700097688
K00001  494.0   198.0   418.0   397.0   403.0   329.0   416.0   126.0   253.0
K00002  16.0    17.0    6.0 32.0    8.0 5.0 3.0 8.0 185.0   15.0    38.0    3.0
K00003  469.0   127.0   413.0   309.0   242.0   314.0   341.0   139.0   208.0
K00004  0.0 0.0 0.0 0.0 0.0 0.0 0.0 15.0    0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
K00005  301.0   99.0    231.0   160.0   157.0   164.0   215.0   42.0    25.0
K00007  0.0 0.0 0.0 0.0 0.0 0.0 0.0 2.0 1.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0
K00008  517.0   83.0    345.0   250.0   236.0   295.0   354.0   16.0    102.0
K00009  10.0    2.0 19.0    13.0    11.0    9.0 54.0    4.0 20.0    9.0 30.0
K00010  6.0 4.0 17.0    25.0    13.0    16.0    100.0   63.0    136.0   12.0
K00011  26.0    0.0 10.0    4.0 2.0 8.0 1.0 0.0 0.0 2.0 10.0    1.0 4.0 0.0 2.0
K00012  318.0   166.0   322.0   302.0   420.0   271.0   371.0   115.0   200.0
K00013  465.0   162.0   397.0   302.0   331.0   270.0   357.0   82.0    191.0
K00014  661.0   164.0   541.0   402.0   427.0   412.0   520.0   198.0   256.0
K00015  0.0 4.0 2.0 26.0    22.0    0.0 0.0 4.0 1.0 0.0 0.0 0.0 0.0 0.0 1.0 0.0
K00016  294.0   49.0    261.0   187.0   173.0   186.0   288.0   116.0   357.0
K00018  94.0    56.0    67.0    88.0    127.0   77.0    140.0   72.0    1.0 107.
K00019  1.0 4.0 3.0 27.0    22.0    1.0 0.0 5.0 47.0    0.0 0.0 0.0 0.0 0.0 1.0
K00020  271.0   103.0   182.0   188.0   162.0   129.0   110.0   46.0    113.0
K00021  0.0 0.0 0.0 0.0 0.0 0.0 0.0 19.0    8.0 0.0 0.0 0.0 0.0 63.0    0.0 0.0
K00022  2.0 4.0 4.0 27.0    22.0    2.0 0.0 10.0    50.0    1.0 0.0 0.0 0.0 0.0 1.0
K00023  1.0 4.0 3.0 27.0    22.0    1.0 0.0 2.0 3.0 0.0 0.0 0.0 0.0 0.0 1.0 0.0
K00024  84.0    113.0   77.0    138.0   171.0   98.0    185.0   52.0    103.0
hmp_ko_subset.tsv
```

Step 2: Predict metagenome contents



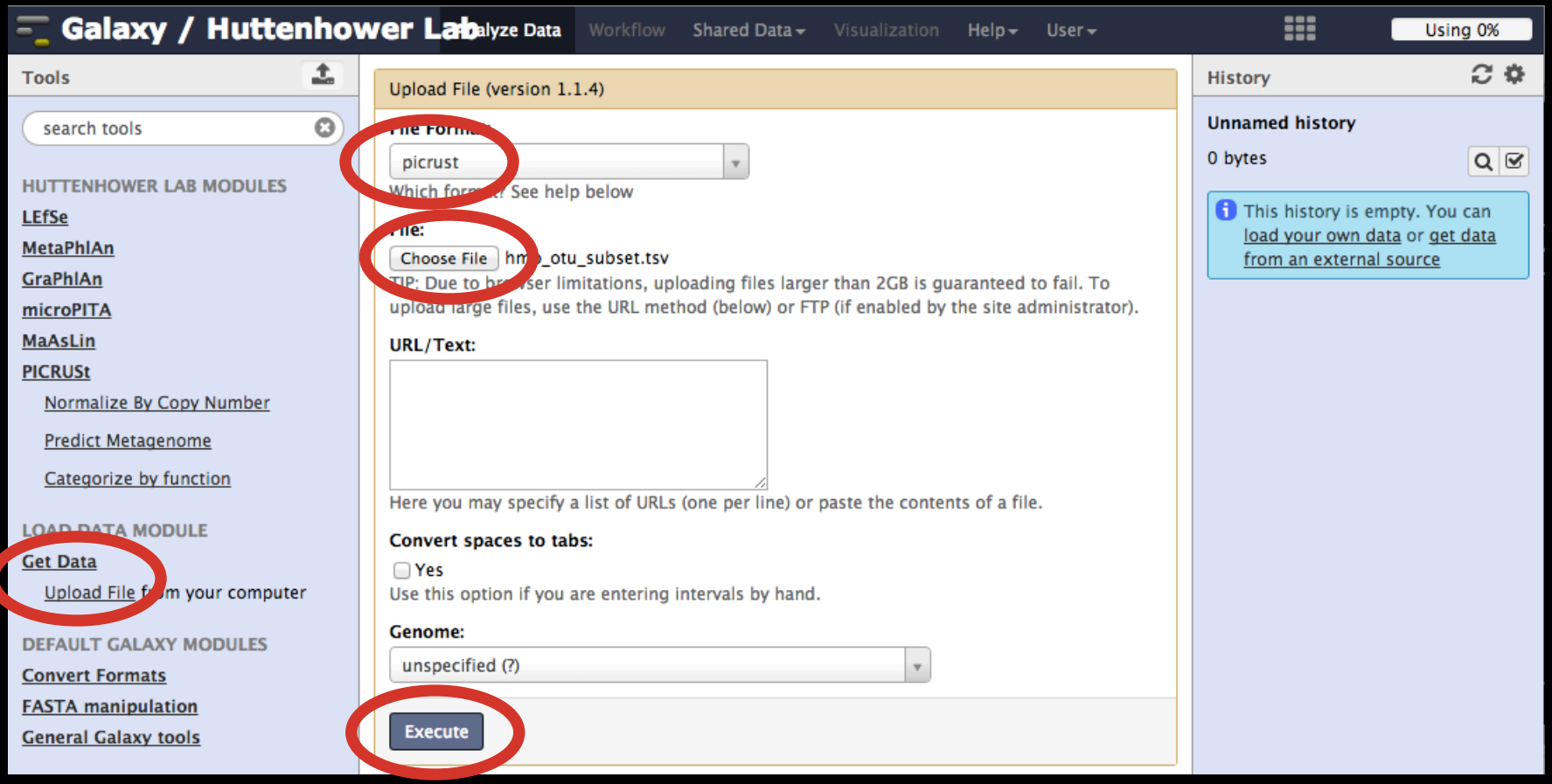
	A	B	C	D	E	F	G	H	I	J
1	# Constructed from biom file									
2	#OTU ID	700098429	700016960	700095467	700101581	700034907	700097688	700098542	700098554	700101242
3	K00001	494	198	418	397	403	329	416	126	253
4	K00002	16	17	6	32	8	5	3	8	185
5	K00003	469	127	413	309	242	314	341	139	208
6	K00004	0	0	0	0	0	0	0	15	0
7	K00005	301	99	231	160	157	164	215	42	25
8	K00007	0	0	0	0	0	0	0	2	1
9	K00008	517	83	345	250	236	295	354	16	102
10	K00009	10	2	19	13	11	9	54	4	20
11	K00010	6	4	17	25	13	16	100	63	136
12	K00011	26	0	10	4	2	8	1	0	0
13	K00012	318	166	322	302	420	271	371	115	200
14	K00013	465	162	397	302	331	270	357	82	191
15	K00014	661	164	541	402	427	412	520	198	256
16	K00015	0	4	2	26	22	0	0	4	1

PICRUSt optional behavior

- You can associate predicted gene abundances with the organism that contains them
  - `metagenome_contributions.py`
- You can show the confidence intervals of all predictions
  - `--with_confidence` argument to `predict_metagenomes.py`
- You can summarize per-gene predictions to per-pathway predictions
  - `categorize_by_function.py`
- You can input a PICRUSt output BIOM file into HUMAnN to reconstruct pathways

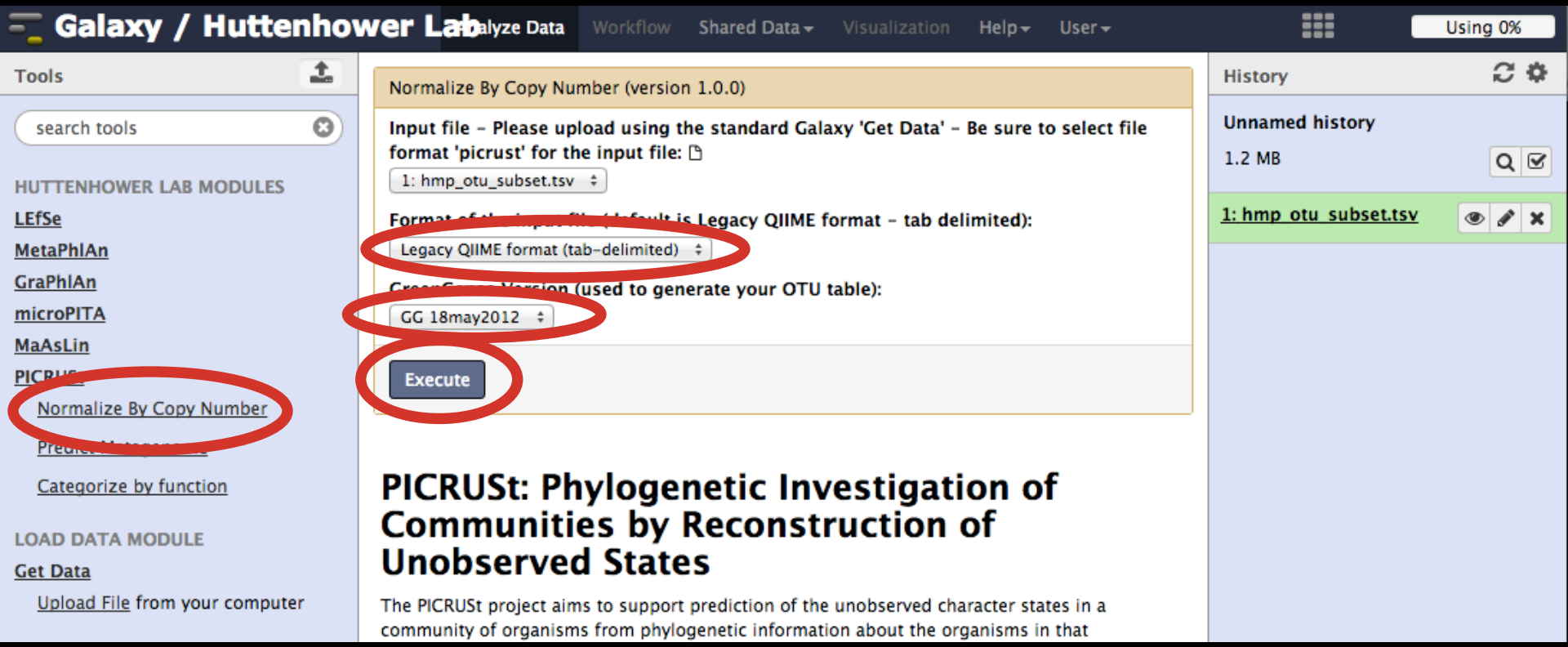
PICRUSt in Galaxy

- http://huttenhower.sph.harvard.edu/picrust
  - First get data!
  - `scp` from `~/workshop_data/metagenomics/biobakery/data/hmp_otu_subset.tsv`



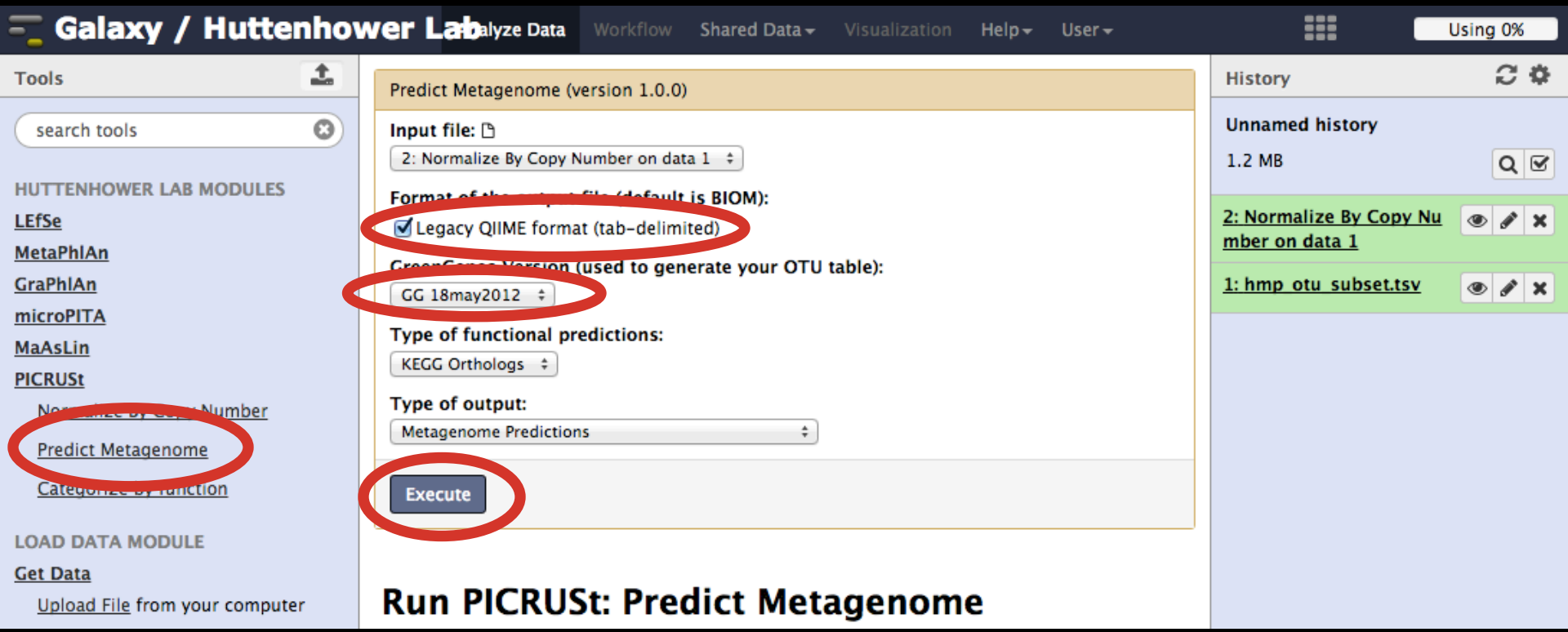
PICRUSt in Galaxy

- Next normalize by copy number



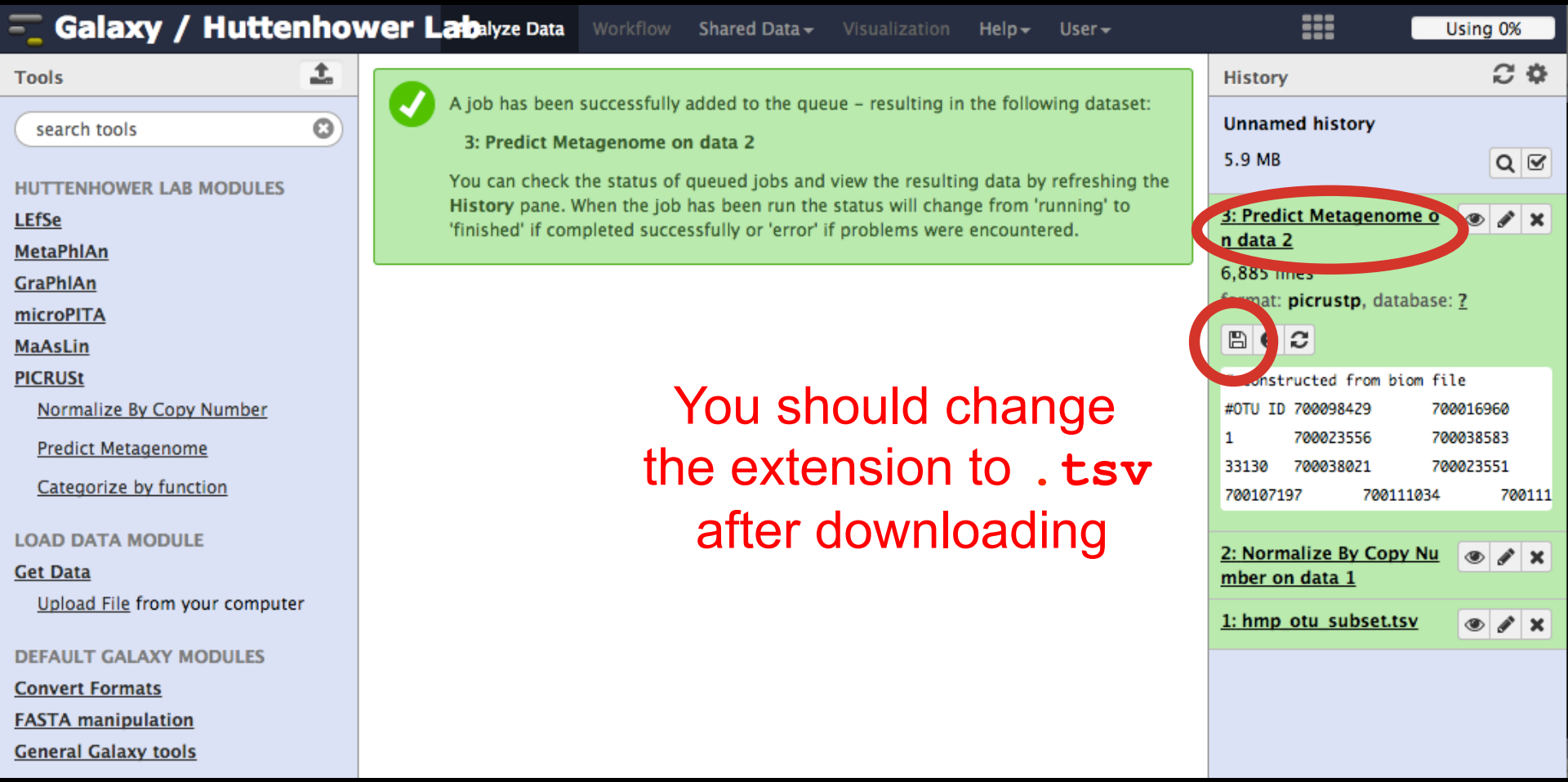
PICRUSt in Galaxy

- Then predict your metagenome

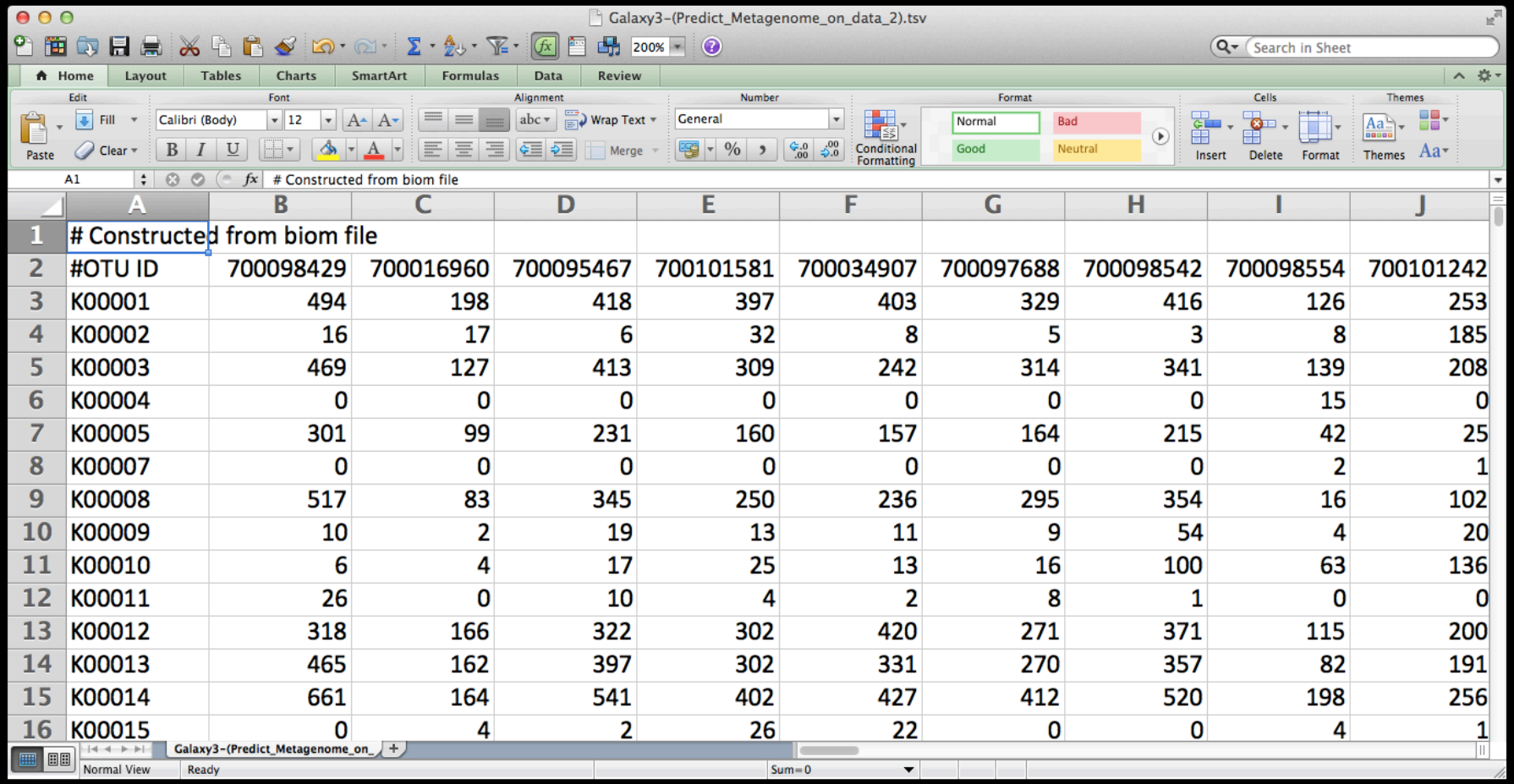


PICRUSt in Galaxy

- You can download the resulting gene table…



PICRUSt in Galaxy



	A	B	C	D	E	F	G	H	I	J
1	# Constructed from biom file									
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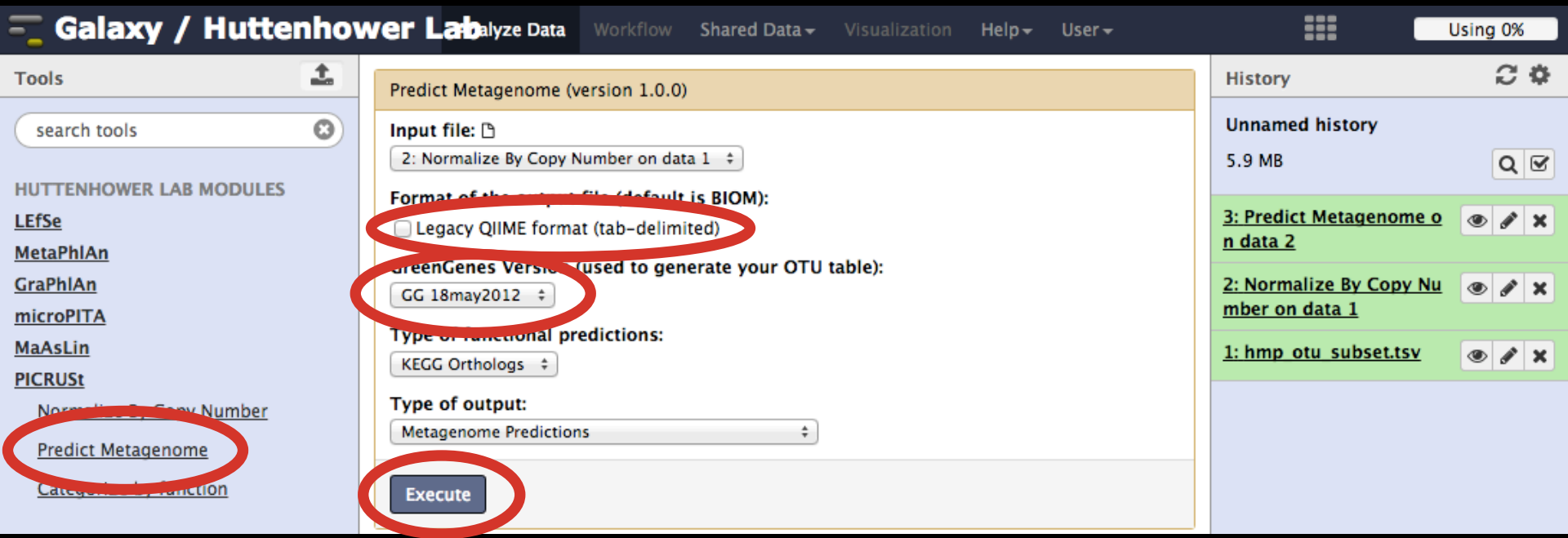
4	K00002	16	17	6	32	8	5	3	8	185
5	K00003	469	127	413	309	242	314	341	139	208
6	K00004	0	0	0	0	0	0	0	15	0
7	K00005	301	99	231	160	157	164	215	42	25
8	K00007	0	0	0	0	0	0	0	2	1
9	K00008	517	83	345	250	236	295	354	16	102
10	K00009	10	2	19	13	11	9	54	4	20
11	K00010	6	4	17	25	13	16	100	63	136
12	K00011	26	0	10	4	2	8	1	0	0
13	K00012	318	166	322	302	420	271	371	115	200
14	K00013	465	162	397	302	331	270	357	82	191
15	K00014	661	164	541	402	427	412	520	198	256
16	K00015	0	4	2	26	22	0	0	4	1

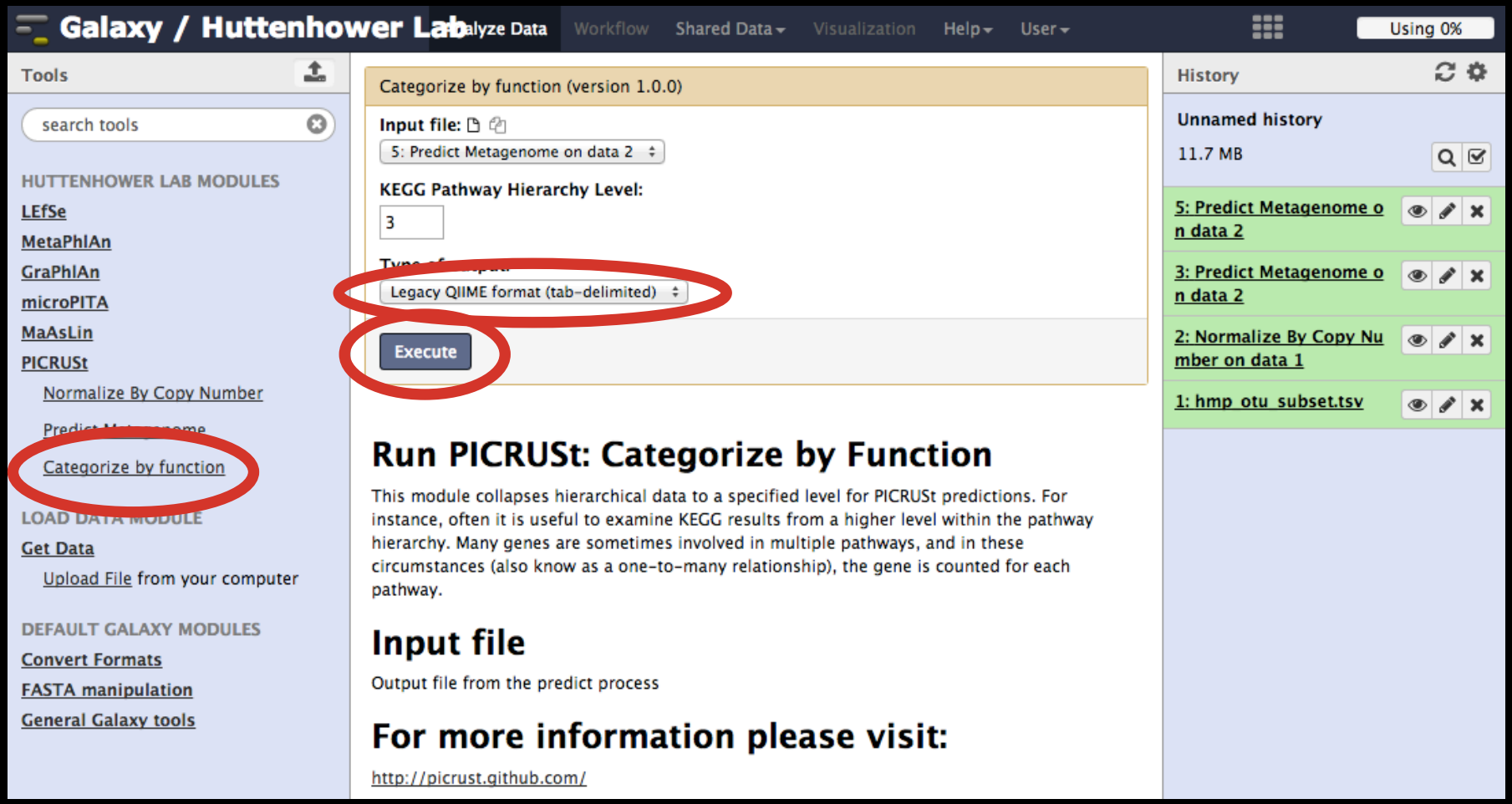
PICRUSt in Galaxy

- …or you can summarize genes to pathways
  - Note that this only works on BIOM files



PICRUSt in Galaxy

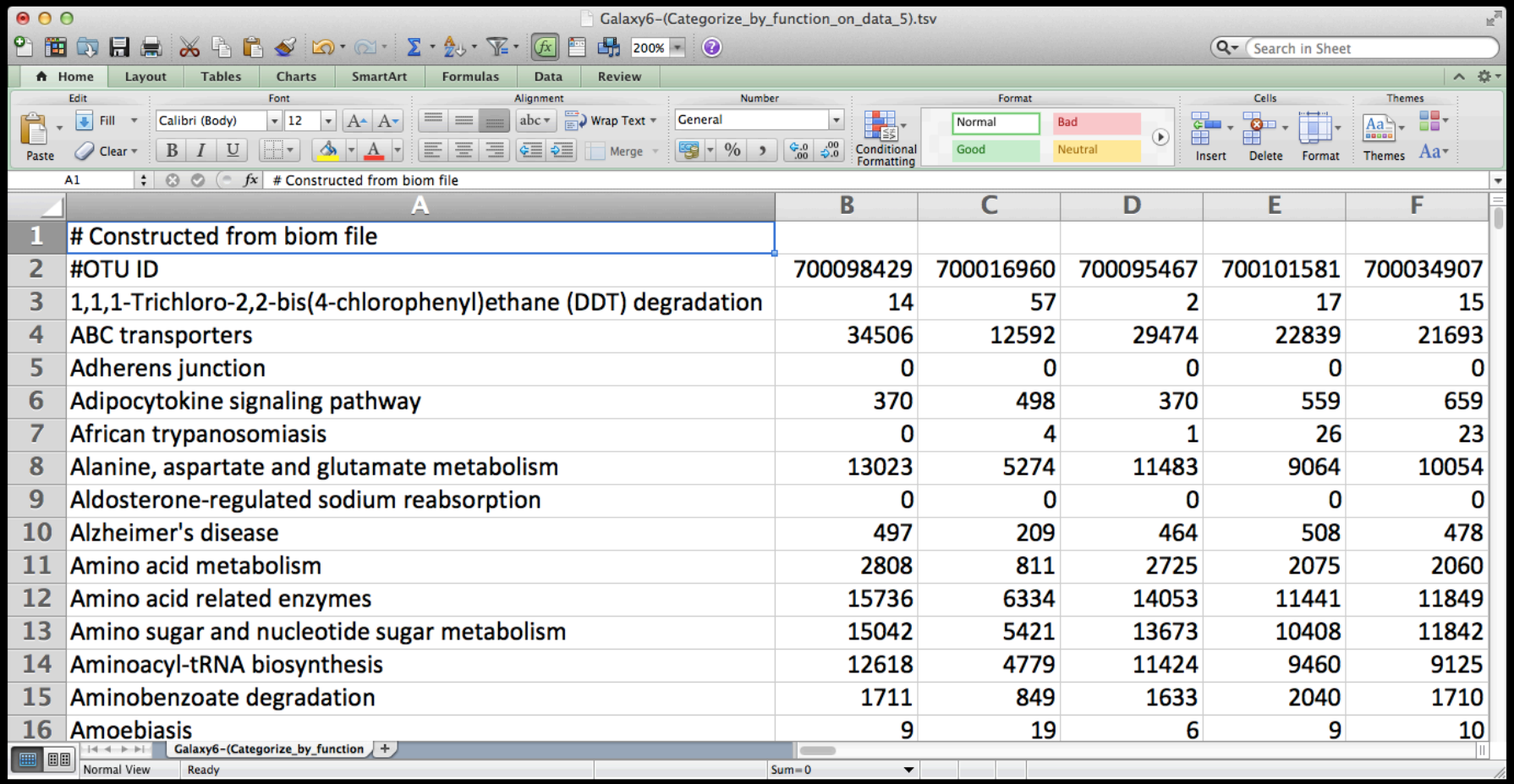
- …continuing where we left off…



PICRUSt in Galaxy

- …and then download them.

PICRUSt in Galaxy



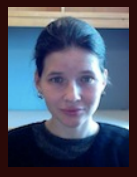
	A	B	C	D	E	F
1	# Constructed from biom file					
2	#OTU ID	700098429	700016960	700095467	700101581	700034907
3	1,1,1-Trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) degradation	14	57	2	17	15
4	ABC transporters	34506	12592	29474	22839	21693
5	Adherens junction	0	0	0	0	0
6	Adipocytokine signaling pathway	370	498	370	559	659
7	African trypanosomiasis	0	4	1	26	23
8	Alanine, aspartate and glutamate metabolism	13023	5274	11483	9064	10054
9	Aldosterone-regulated sodium reabsorption	0	0	0	0	0
10	Alzheimer's disease	497	209	464	508	478
11	Amino acid metabolism	2808	811	2725	2075	2060
12	Amino acid related enzymes	15736	6334	14053	11441	11849
13	Amino sugar and nucleotide sugar metabolism	15042	5421	13673	10408	11842
14	Aminoacyl-tRNA biosynthesis	12618	4779	11424	9460	9125
15	Aminobenzoate degradation	1711	849	1633	2040	1710
16	Amoebiasis	9	19	6	9	10

Thanks!

http://huttenhower.sph.harvard.edu


Alex Kostic


Levi Waldron


Xochitl Morgan


Tim Tickle


Daniela Boernigen


Lauren McIver

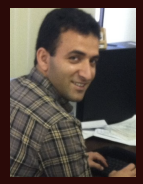

George Weingart


Emma Schwager


Eric Franzosa


Boyu Ren


Tiffany Hsu


Ali Rahnavard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Jim Kaminski


Regina Joice


Koji Yasuda


Kevin Oh


Galeb Abu-Ali


Afrah Shafquat


Randall Schwager


Chengwei Luo


Keith Bayer


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