# Meta'omic functional profiling with ShortBRED

Curtis Huttenhower

09-19-15

Harvard School of Public Health
Department of Biostatistics

U. Oregon

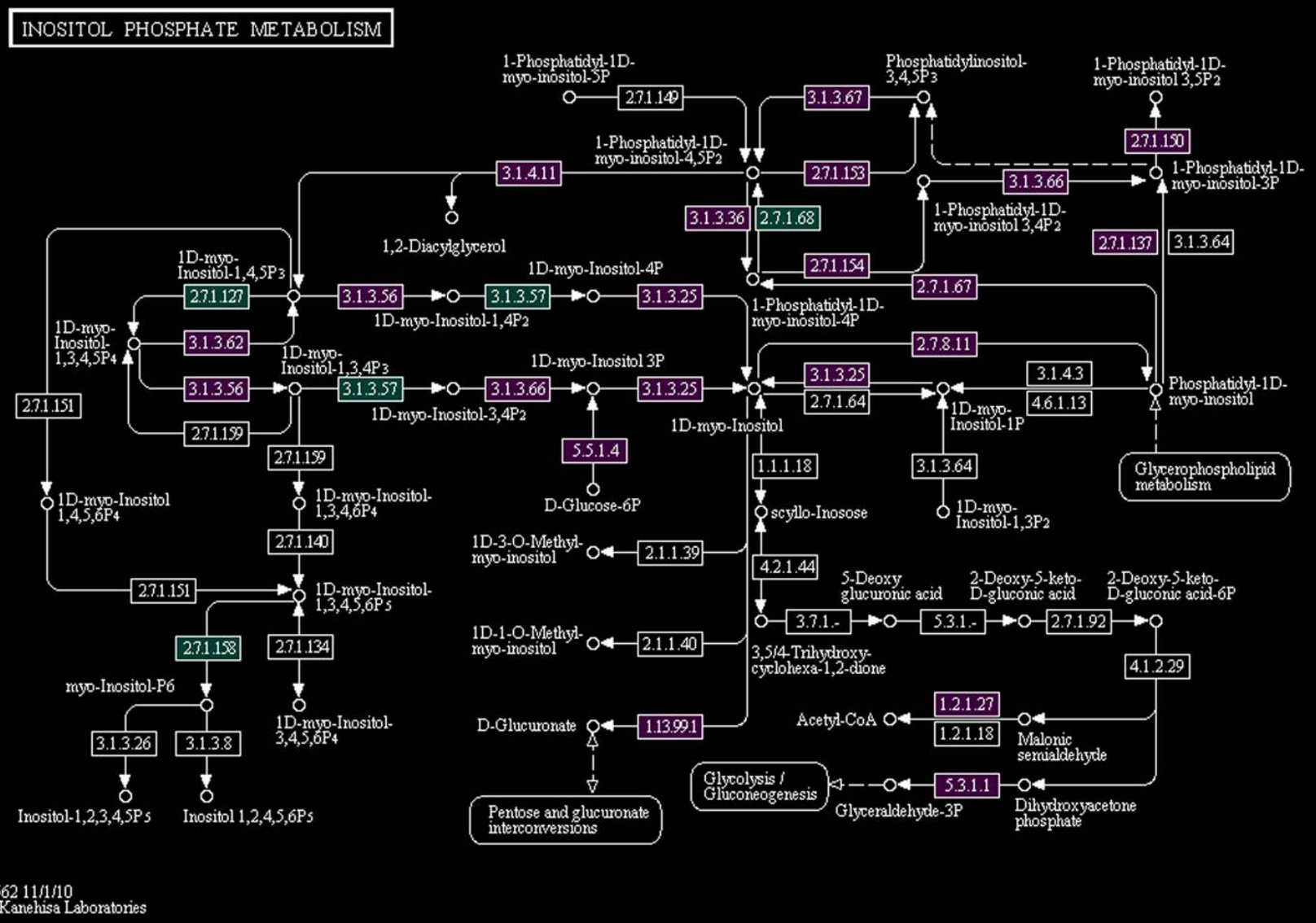
BROAD INSTITUTE

# The two big questions…

**Who is there?**
(taxonomic profiling)

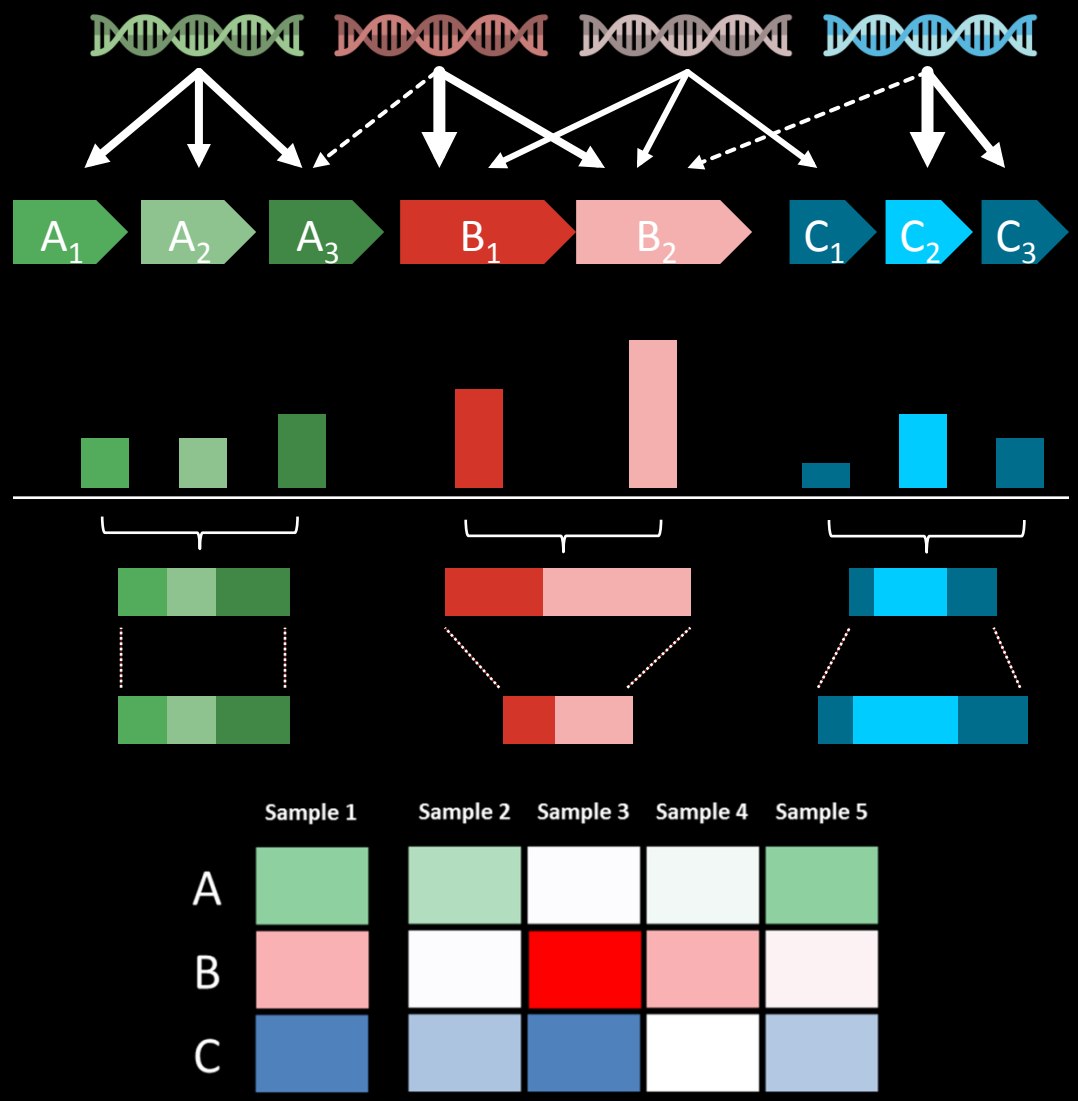
**What are they doing?**
(functional profiling)

# (What we mean by "function")



# HUMAnN

## *HMP Unified Metabolic Analysis Network*




Short reads + protein families

Translated BLAST search

$$c(g) = \frac{1}{|g|} \sum_{r} \frac{\sum_{a(r)} (1 - p_a)\Delta(a = g)}{\sum_{a(r)} 1 - p_a}$$

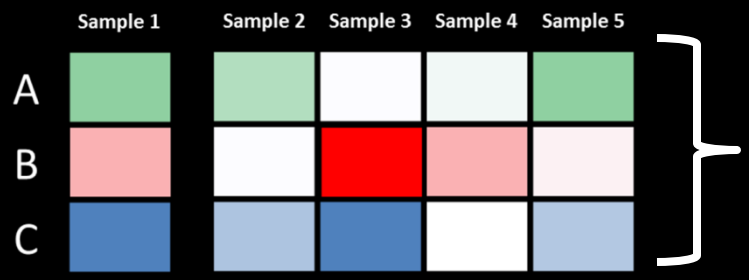
Weight hits by significance

Sum over families

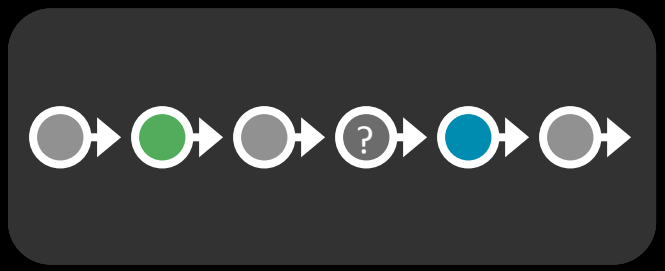
Adjust for sequence length

Repeat for each metagenomic or metatranscriptomic sample

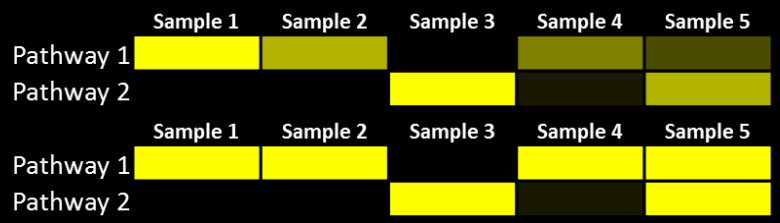
# HUMAnN
## *HMP Unified Metabolic Analysis Network*




Millions of hits are collapsed into thousands of gene families (KOs) (*still a large number*)

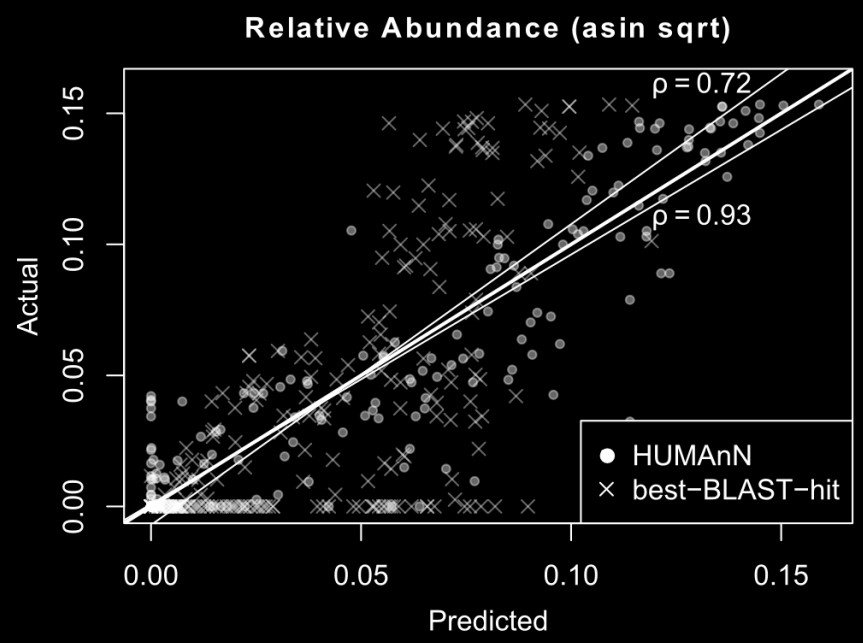


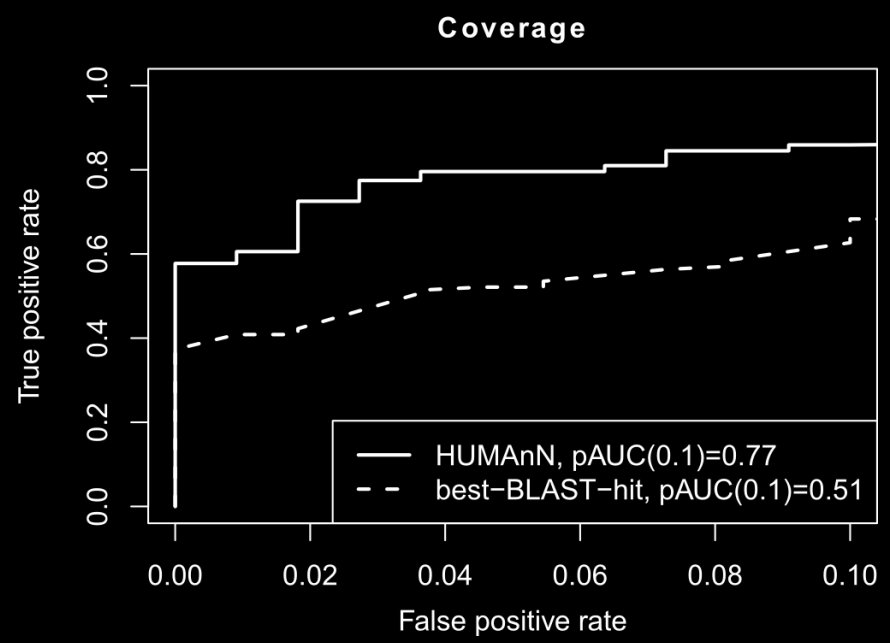

- Map genes to KEGG ~~pathways~~ modules
- Use MinPath (Ye 2009) to find simplest pathway explanation for observed genes
- Remove pathways unlikely to be present due to low organismal abundance
- Smooth/fill gaps




Collapsing KO abundance into KEGG module abundance (or presence/absence) yields a smaller, more tractable feature set

# HUMAnN accuracy

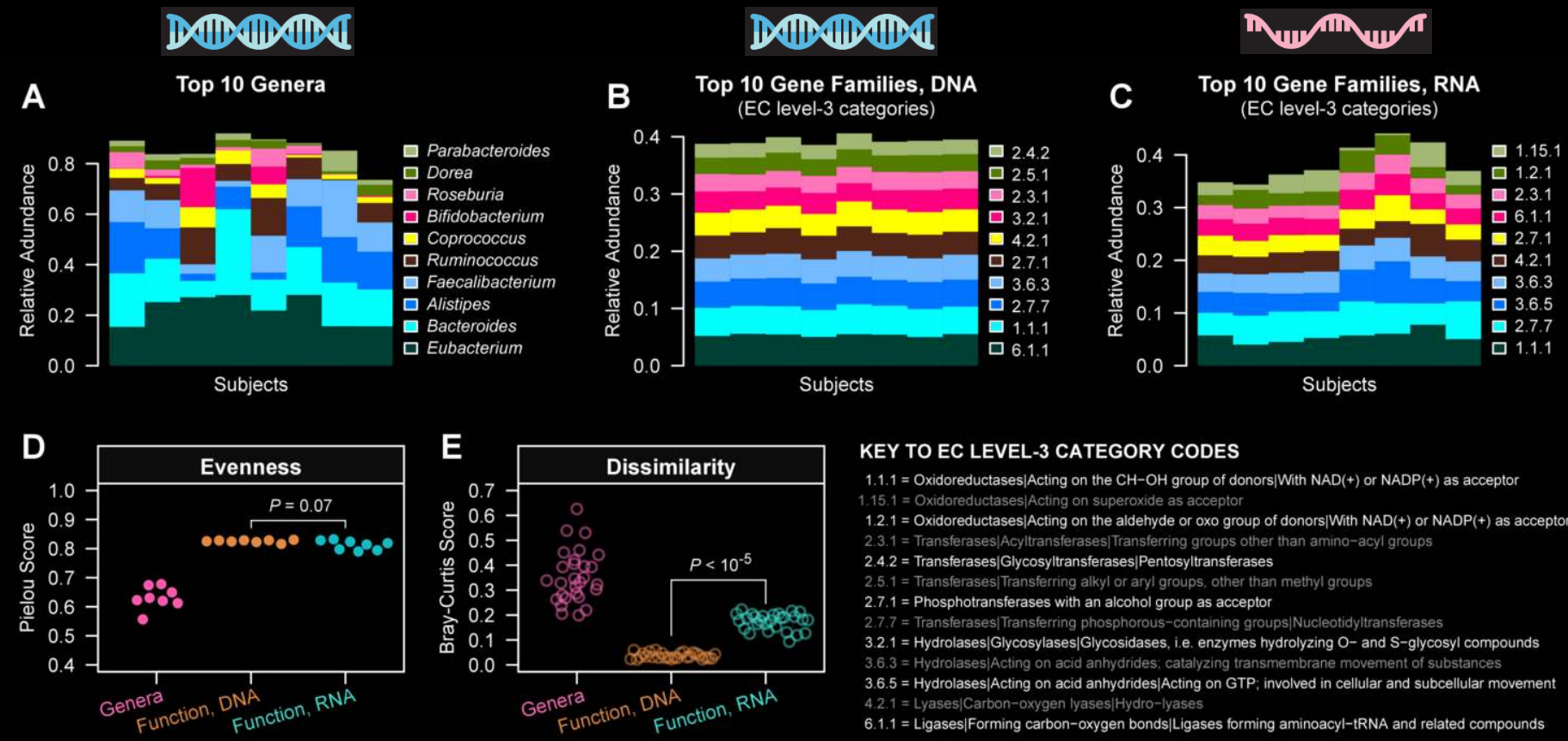



Validated against synthetic metagenome samples (similar to MetaPhlAn validation)

Gene family abundance and pathway presence/absence calls beat naïve best-BLAST-hit strategy

# HUMAnN in action



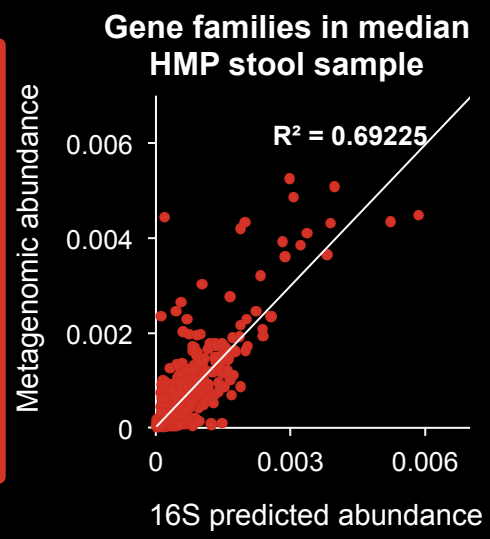

Franzosa et al. *PNAS* 11:E2329-38 (2014)

# **PICRUSt:** Inferring community metagenomic potential from marker gene sequencing

With Rob Knight, Rob Beiko

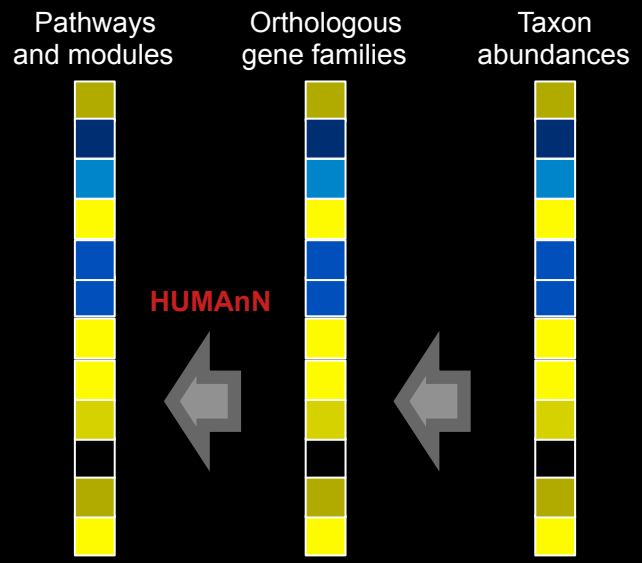
One can recover ***general*** community function with reasonable accuracy from 16S profiles.

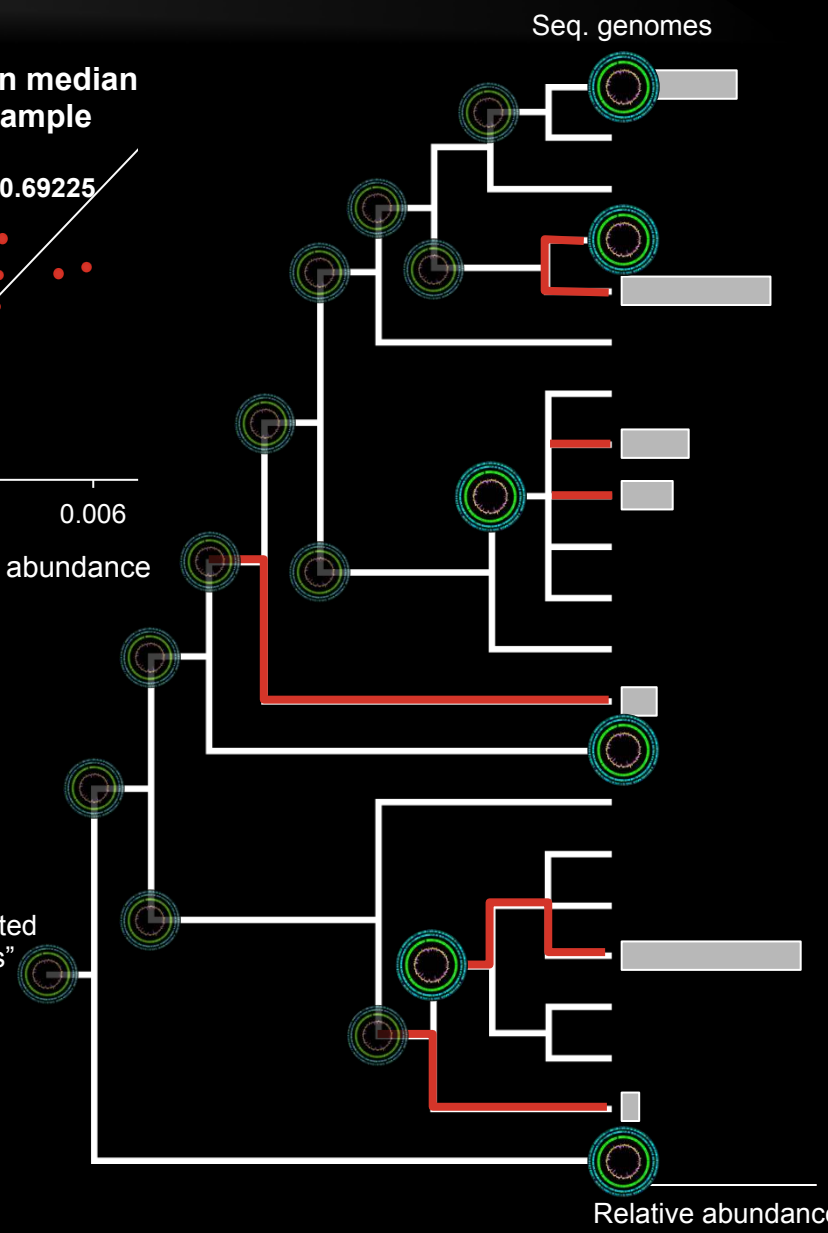
http://picrust.github.com



Jesse Zaneveld

Morgan Langille



# What's there: ShortBRED

Jim Kaminski

- **ShortBRED** is a tool for quantifying protein families in metagenomes
  - Short Better REad Dataset

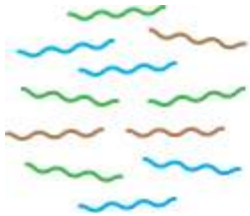
- Inputs:
  - FASTA file of proteins of interest
  - Large reference database of protein sequences (FASTA or blastdb)
  - Metagenomes (FASTA/FASTQ nucleotide files)
- Outputs:
  - Short, unique markers for protein families of interest (FASTA)
  - Relative abundances of protein families of interest in each metagenome (text file, RPKM)

- Compared to BLAST (or HUMAnN), this is:
  - Faster
  - More specific

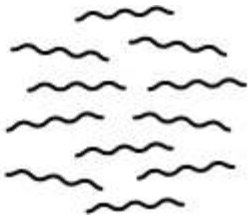
# What's there: ShortBRED algorithm

- Cluster proteins of interest into families
  - Record consensus sequences
- Identify and common areas among proteins
  - Compared against each other
  - Compared against reference database
  - Remove all of these
- Remaining subseqs. uniquely ID a family
  - Record these as markers for that family

# What's there: ShortBRED marker identification



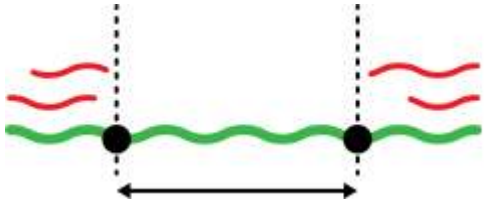
**Prots of interest**



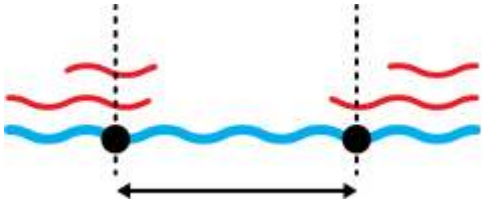
**Reference database**

**Cluster into families**

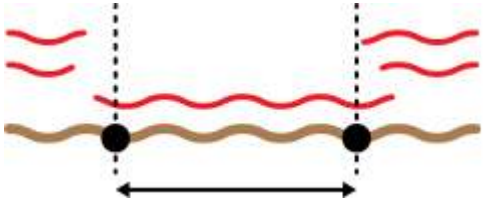
**Identify short, common regions**



**True Marker**

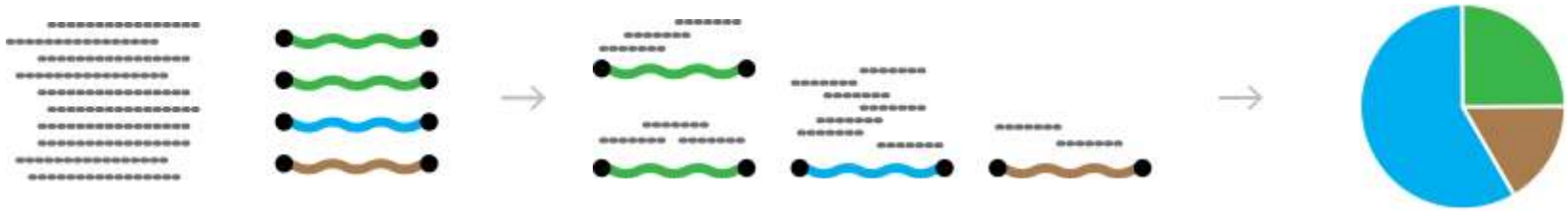


**Junction Marker**



**Quasi Marker**

# What's there: ShortBRED family quantification



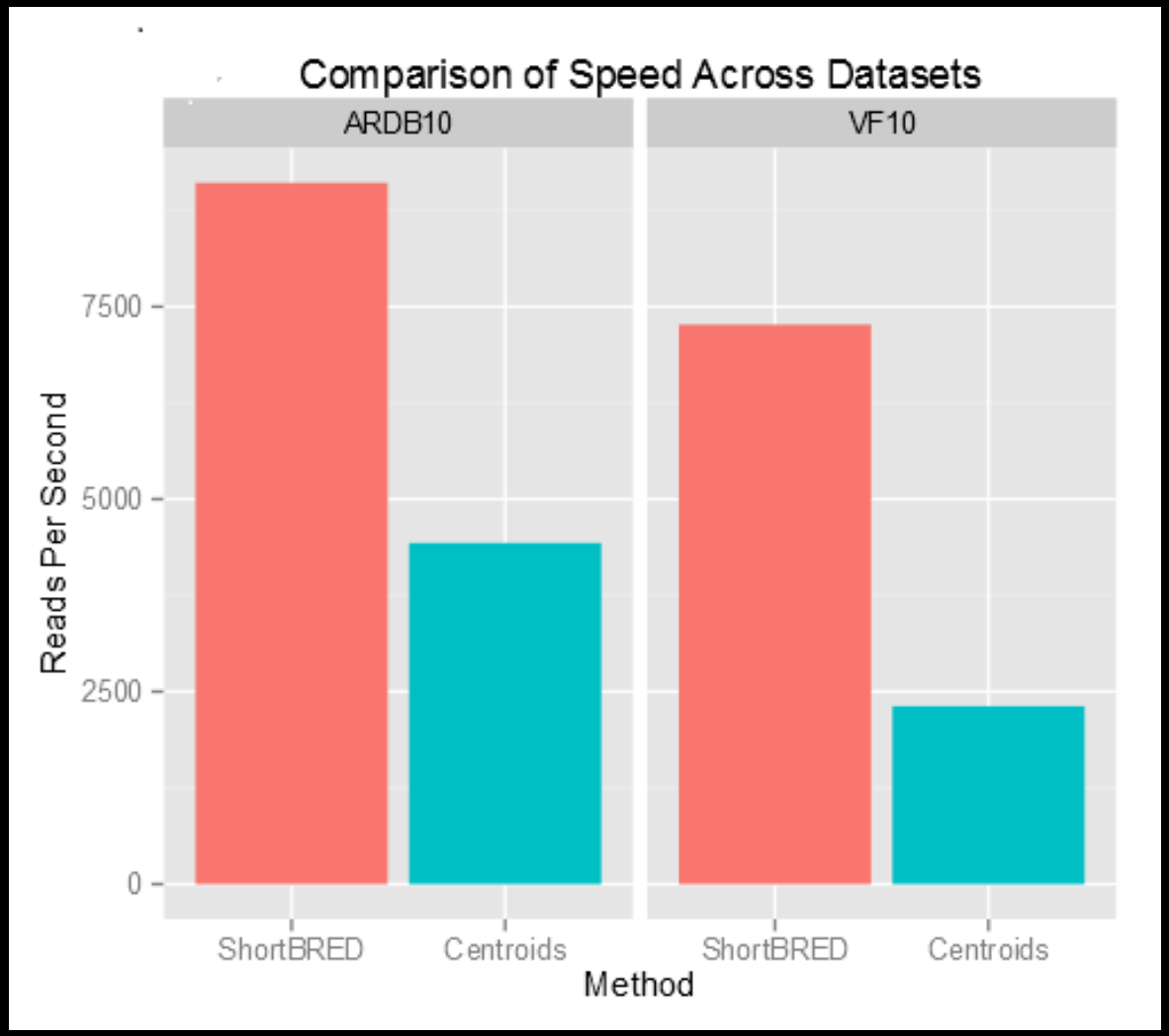
**Metagenome reads**

**ShortBRED markers**

**Translated search for high ID hits**

**Normalize relative abundances**

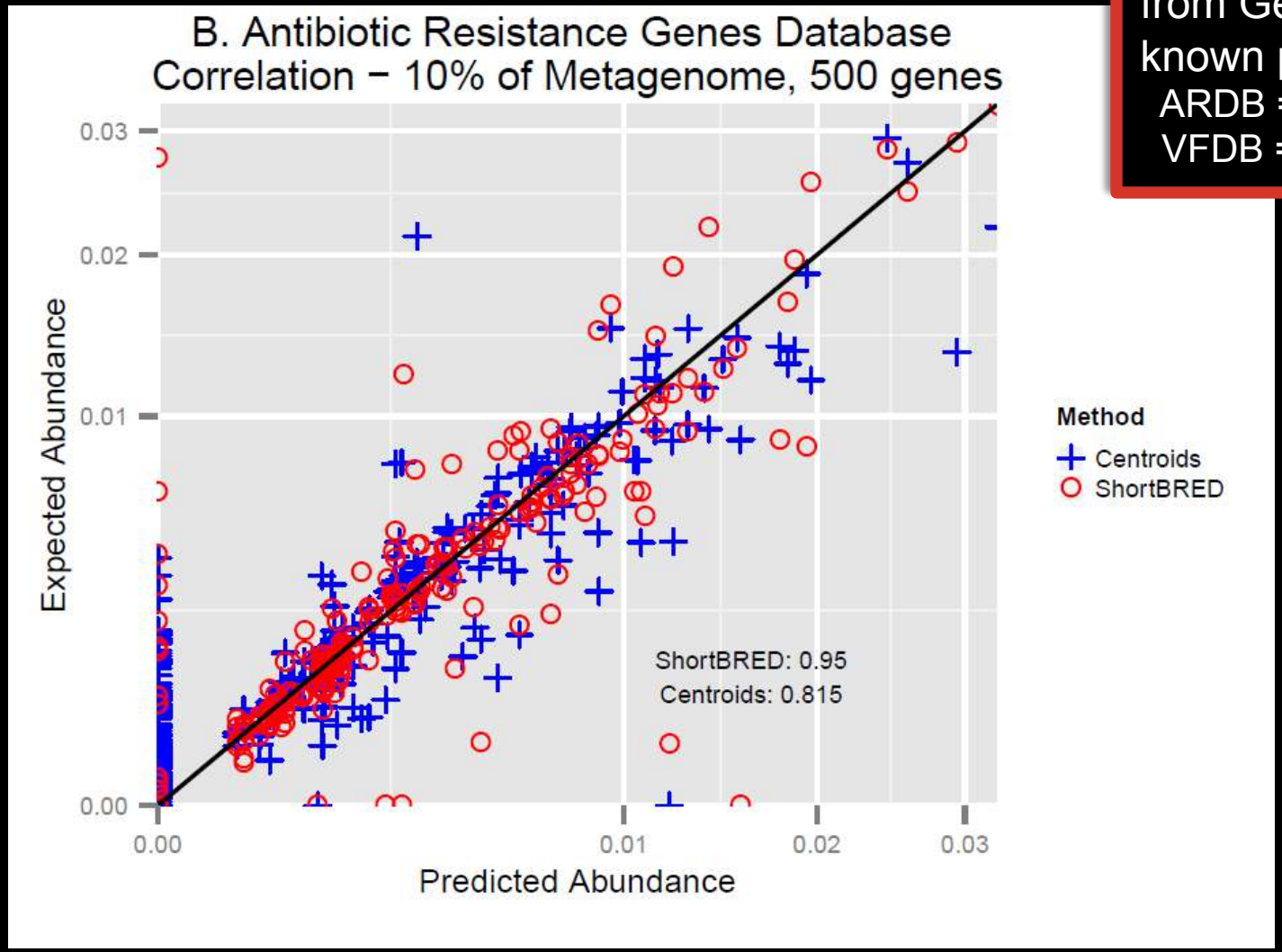
# What's there: ShortBRED's fast




Six synthetic metagenomes from GemSim, spiked with known proteins of interest:
ARDB = Antibiotic Resistance
VFDB = Virulence Factors

# What's there: ShortBRED's accurate

Six synthetic metagenomes from GemSim, spiked with known proteins of interest:
ARDB = Antibiotic Resistance
VFDB = Virulence Factors



# 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`**.

# What's there: ShortBRED

- ShortBRED is available at [http://huttenhower.sph.harvard.edu/shortbred](http://huttenhower.sph.harvard.edu/shortbred)

**The Huttenhower Lab**

Department of Biostatistics, Harvard School of Public Health

Contact | Documentation | People | Presentations | Publications | Research | Teaching

Home

You *could* download ShortBRED by clicking here

## ShortBRED

ShortBRED, the Short Better REad Dataset, is a method for high-precision detection and quantification of functional protein families in microbial communities (metagenomes and metatranscriptomes). It considers a set of protein sequences of interest, reduces them to a set of unique identifying strings ("markers"), and then searches for these markers in metagenomes or metatranscriptomes to very precisely determine the presence and abundance of the original protein families. ShortBRED-Identify clusters the protein sequences into families, removes regions of overlap among the consensus sequences and between the consensus sequences and a set of reference proteins, and saves the remaining sequences as high-confidence unique markers for the families. ShortBRED-Quantify then searches for the markers in unassembled shotgun meta'omic data and returns a normalized relative abundance table of the protein families found in the data.

**For more information on the technical aspects to this program, or to cite ShortBRED, please reference the following manuscript:**

Kaminski J, Gibson M, Franzosa E, Segata N, Dantas G, and Huttenhower C. Fast and accurate meta'omic search with ShortBRED. (In progress)

### Download ShortBRED (preliminary version)

Please note that this is a beta version of ShortBRED. An official release will be ready soon.

Download ShortBRED here

You may also install ShortBRED using Mercurial:

```
$ hg clone https://bitbucket.org/biobakery/shortbred
```

More information on the ShortBRED implementation, including runtime documentation, is available at its **Bitbucket page**.

# From the command line...

- But don't!
  - Instead, we've installed ShortBRED already for you
- To see what you can do, run:

```
shortbred_identify.py -h | less
shortbred_quantify.py -h | less
```

```
1. ssh
usage: shortbred_identify.py [-h] [--goi SGOIPROTS] [--ref SREFPROTS]
                             [--refdb DIRREFDB] [--goiblast SGOIBLAST]
                             [--refblast SREFBLAST] [--goiclust SCLUST]
                             [--map_in SMAPIN] [--markers SMARKERS]
                             [--map_out SMAP] [--clustid DCLUSTID]
                             [--qclustid DQCLUSTID] [--consthresh DCONSTHRESH]
                             [--threads ITHREADS] [--id DID] [--len DL]
                             [--minAln ILENMIN] [--markerlength IMLENGTH]
                             [--totlength ITOTLENGTH] [--qthresh ITHRESH]
                             [--qmlength IQMLENGTH] [--xlimit IXLIMIT]
                             [--tmpdir STMP] [--usearch STRUSEARCH]
                             [--muscle STRMUSCLE] [--cdhit STRCDHIT]
                             [--blastp STRBLASTP]

ShortBRED Identify:
 This program produces a set of markers for your proteins of interest.
 The minimum input files required to run the program are:
        [--goi] 1) A fasta file of proteins, for which you want to build marker
s.
        [--ref] 2) A fasta file of reference proteins
 The program will output a file fasta file of markers [--markers].

Example:
        $ ./ python shortbred_identify.py --goi example/input_prots.faa --ref ex
:
```

# Getting some annotated protein sequences

You could download the ARDB protein sequences here

- Go to http://ardb.cbcb.umd.edu

## ARDB - Antibiotic Resistance Genes Database

HOME | DOCUMENTATION | BLAST | ADVANCED SEARCH | BROWSE

Database [All Databases] Input [ ] Search Help Tutorial for ARDB

**Antibiotic Resistance**

Brief introduction to antibioitc resistance.

**Analysis & Tools**

- Single Gene Annotation
- Genome Annotation and Comparision
- Genome Resistance Profiles Comparison
- Mutation Detection

**GO Annotation**

How to use GO terms to annotate resistance genes?

### Welcome to Antibiotic Resistance Genes Database Home Page

Our motivations in creating ARDB are to:

- provide a centralized compendium of information on antibiotic resistance
- facilitate the consistent annotation of resistance information in newly sequenced organisms
- facilitate the identification and characterization of new genes

More...

### News

ARDB is not being maintained at the moment, though we hope to secure funding to further improve it. All underlying data are available for download at: ftp://ftp.cbcb.umd.edu/pub/data/ARDB/ARDBflatFiles.tar.gz. Documentation about the provided data is available at ftp://ftp.cbcb.umd.edu/pub/data/ARDB/doc4ARDBflatFiles.pdf.

ARDB is recently updated to Version 1.1 on July 3, 2009.

**Database Statistics**
Version: 1.1
Last Update: July 3, 2009

Genes: 23137

Types: 380

Antibiotics: 249

Genomes: 632

Species: 1737

Genera: 267

Vectors, Plasmids: 2881

# From the command line...

- But don't!
  - Instead, we've downloaded the important file for you
- Take a look by running:

```
less ~/workshop_data/metagenomics/biobakery/data/resisGenes.pfasta
```

1. screen (less)

```
>ZP_02959935 hypothetical protein PROSTU_01837 [Providencia stuartii ATCC 25827]
MGIEYRSLHTSQLTLSEKEALYDLLIEGFEGDFSHDDFAHTLGGMHVMAFDQQKLVGHVA
IIQRHMALDNTPISVGYVEAMVVEQSYRRQGIGRQLMLQTNKIIASCYQLGLLSASDDGQ
KLYHSVGWQIWKGKLFELKQGSYIRSIEEEGGVMGWKADGEVDFTASLYCDFRGGDQW
>Q52424 RecName: Full=Aminoglycoside 2'-N-acetyltransferase; AltName: Full=AAC(2
MGIEYRSLHTSQLTLSEKEALYDLLIEGFEGDFSHDDFAHTLGGMHVMAFDQQKLVGHVA
IIQRHMALDNTPISVGYVEAMVVEQSYRRQGIGRQLMLQTNKIIASCYQLGLLSASDDGQ
KLYHSVGWQIWKGKLFELKQGSYIRSIEEEGGVMGWKADGEVDFTASLYCDFRGGDQW
>AAA03550 aminoglycoside 2'-N-acetyltransferase [Providencia stuartii].
MGIEYRSLHTSQLTLSEKEALYDLLIEGFEGDFSHDDFAHTLGGMHVMAFDQQKLVGHVA
IIQRHMALDNTPISVGYVEAMVVEQSYRRQGIGRQLMLQTNKIIASCYQLGLLSASDDGQ
KLYHSVGWQIWKGKLFELKQGSYIRSIEEEGGVMGWKADGEVDFTASLYCDFRGGDQW
>Q49157 RecName: Full=Aminoglycoside 2'-N-acetyltransferase; AltName: Full=AAC(2
MPFQDVSAPVRGGILHTARLVHTSDLDQETREGARRMVIEAFEGDFSDADWEHALGGMHA
FICHHGALIAHAAVVQRRLLYRDTALRCGYVEAVAVREDWRGQGLATAVMDAVEQVLRGA
YQLGALSASDTARGMYLSRGWLPWQGPTSVLQPAGVTRTPEDDEGLFVLPVGLPAGMELD
TTAEITCDWRDGDVW
>NP_214776 aminoglycoside 2'-N-acetyltransferase AAC (AAC(2')-IC) [Mycobacterium
MHTQVHTARLVHTADLDSETRQDIRQMVTGAFAGDFTETDWEHTLGGMHALIWHHGAIIA
HAAVIQRRLIYRGNALRCGYVEGVAVRADWRGQRLVSALLDAVEQVMRGAYQLGALSSSA
RARRLYASRGWLPWHGPTSVLAPTGPVRTPDDDGTVFVLPIDISLDTSAELMCDWRAGDV
W
>NP_334681 aminoglycoside 2-N-acetyltransferase [Mycobacterium tuberculosis CDC1
MHTQVHTARLVHTADLDSETRQDIRQMVTGAFAGDFTETDWEHTLGGMHALIWHHGAIIA
ttenh/Dropbox/shared/ShortBRED/data/ARDB/ardbAnno1.0/blastdb/resisGenes.pfasta
```

# Getting some reference protein sequences

- Go to http://metaref.org

Home Abou Download Help

Keyword Search Help

Microbial taxonomy Go

You could download the MetaRef protein sequences here

Browse

Bacteria: 2706 Genomes

Archaea: 112 Genomes

Taxonomy Correction Info

Highlighted Clades

(Commonly Found in Human Microbiome)

Airways Nares

*Corynebacterium accolens*

*Propionibacterium acnes*

*Staphylo. epidermidis*

Buccal Mucosa

*Gemella haemolysans*

*Haemophilus influenzae*

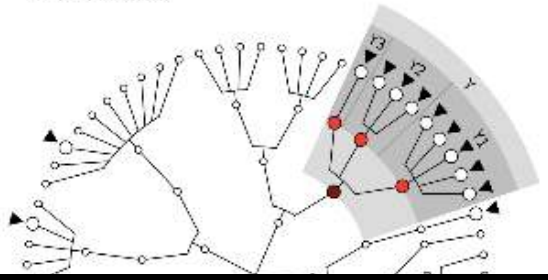
*Streptococcus mitis*

## MetaRef Database v 1.0

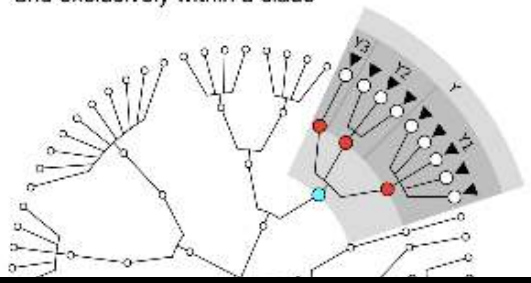
MetaRef is a resource to comprehensively catalog and characterize clade-specific microbial genes. We identify and provide all core genes associated with all microbial species and genera with available reference genomes (final or draft). A subset of these gene families are consistently present in one or more taxonomic clades, which allows us to further indicate them as marker genes.

MetaRef paper is now available on PubMed.

**Core families**: genes present consistently within a clade



**Marker families**: genes present consistently and exclusively within a clade



# Running ShortBRED-Identify

- But don't!
  - We'll use an example mini reference database for speed
- Lets make some antibiotic resistance markers by running:

```
ln -s /usr/bin/cdhit /home/ubuntu/Programs/cd-hit
shortbred_identify.py
  --goi ~/workshop_data/metagenomics/biobakery/data/resisGenes.pfasta
  --ref ~/workshop_data/metagenomics/biobakery/software/shortbred/example/ref_prots.faa
  --markers ardb_markers.faa
less ardb_markers.faa
```

  - This should take ~5 minutes
    - If you get bored waiting, kill it and copy:

```
~/workshop_data/metagenomics/biobakery/results/shortbred/ardb_markers.faa
```

  - It will produce lots of status output as it runs

# ShortBRED markers

3. [screen 3: bash] chuttenhower@class:/class/stamps-software/biobake...

```
>AAY52010_TM_#01
ISILILCRVML
>AAY52010_TM_#02
DKQIELSAEM
>AAY52010_TM_#03
KLNTLKRTLEKRE
>AAY52010_TM_#04
VVMYLAHDIKTPLTS
>AAY52010_TM_#05
LLDEAPDMP
>AAY52010_TM_#06
KAYRLEQLID
>AAY52010_TM_#07
IDLYYMLVQM
>AAY52010_TM_#08
DKLARVFNNIL
>AAY52010_TM_#09
IFEKFYRLD
>AAY52010_TM_#10
HGGQIYAESN
>AAD51345_TM_#01
AVSLLGLLAILILPVDR
>AAD51345_TM_#02
IRATYTGASSQTVENAVTQVIEQSQQSLDHLMYMTSTSASDGSAQVNLVFAT
ardb_markers.faa
```

True Markers
at the top

# ShortBRED markers

3. [screen 3: bash] chuttenhower@class:/class/stamps-software/biobake...

```
>AAA25688_TM_#02
PTQLNKGLGTRLVRALVELLFSDPTVTKIQTDPTPNNH
>YP_277581_TM_#01
MSMIYITLNIIAYVIDVRSLILDVRRLVFS
>YP_277581_TM_#02
NILNCMDDSVIAFTVIIQLGAILSITKIFWSQLYGMSMICIKKIFFKQHDDHNHLCIRHI
FLGTFPGIMLGMIFYEKIGLIFELTYIMYGLIIGGIFLLVGELCASKEPRVSRINNITYL
>YP_277581_TM_#03
FSRAGATIGGGLVVGLDRRISS
>YP_277581_TM_#04
SAVLTLYHYRSCIGLMDVLLLIAGSATAFFIALFTVRYFLKIVKNVSLIPFAIYRFLLAG
GIYWGLMT
>1112175A_JM_#01__[1112175A_w=0.486,YP_001103000_w=0.143,YP_001103000_w=0.371]
LFEWEFVEKVDSAIMRLRRRAEPLLEGAALERYE
>1112175A_JM_#02__[1112175A_w=0.515,YP_001103000_w=0.333,YP_001103000_w=0.152]
RKYPRRRVEAAFDHAGVGGGAVVAYVRPEQWLRL
>ABF69686_JM_#01__[ABF69686_w=0.459,ABN80187_w=0.135,ZP_03989103_w=0.405]
DTAYPGEIVILADDTLKLNDILGNEKLLPHKTRI
>YP_002081505_JM_#01__[YP_002081505_w=0.630,YP_274481_w=0.370]
LGTIGGFRLQIEDRGNX
>YP_274481_QM33_#01__[YP_274481_w=0.500,YP_002081505_w=0.500]
PAAFISGLTGQFYKQFALTIAISTVISAFNSLT
>ZP_01817983_JM_#01__[ZP_01817983_w=0.493,YP_001694417_w=0.362,YP_001694417_w=0.
TLTGPFIGGFIKEDFQPVAKEKAIPTKELFTSVK
(END)
```

Junction/Quasi Markers
at the bottom

# Running ShortBRED-Quantify

- Using your existing HMP data subset, you can search for antibiotic resistance proteins in the oral cavity by running:

```
shortbred_quantify.py
  --markers ardb_markers.faa
  --wgs 763577454-SRS014472-Buccal_mucosa.fasta
  --results 763577454-SRS014472-Buccal_mucosa-ARDB.txt
less 763577454-SRS014472-Buccal_mucosa-ARDB.txt
```

  – This should just a few seconds
  – It will again produce lots of status output as it runs

# ShortBRED marker quantification

3. [screen 3: bash] chuttenhower@class:/class/stamps-software/biobake...

```
Family  Count   Hits    TotMarkerLength
YP_001694417    2380.9523809523807  1   26
ZP_04679156 0.0 0   235
ZP_04657259 0.0 0   178
ZP_04635798 0.0 0   144
ZP_04635523 0.0 0   182
ZP_04633951 0.0 0   70
ZP_04616832 0.0 0   9
ZP_04613685 0.0 0   95
ZP_04606269 0.0 0   193
ZP_04577926 0.0 0   185
ZP_04543635 0.0 0   173
ZP_04543532 0.0 0   194
ZP_04433866 0.0 0   205
ZP_04431003 0.0 0   105
ZP_04405580 0.0 0   169
ZP_04405450 0.0 0   300
ZP_04309403 0.0 0   167
ZP_04284182 0.0 0   212
ZP_04244950 0.0 0   51
ZP_04210257 0.0 0   169
ZP_04197552 0.0 0   154
ZP_04175489 0.0 0   70
ZP_04174269 0.0 0   21
tmp.txt
```

RPKMs and raw hit count

Other columns are <u>family name</u> and <u>total AAs among all family makers</u>

# AR proteins in the human gut

- That's boring! Let's get some real data
- `scp` the file to your own computer (optional):

```
~/workshop_data/metagenomics/biobakery/data/shortbred_ardb_hmp_t2d.tsv
```

- This is the result of running:
  - ShortBRED-Identify on the real ARDB + reference
  - ShortBRED-Quantify on the real HMP + T2D data (Qin Nature 2014)
  - Summing each sample's RPKMs for families in each ARDB resistance class

# AR proteins in the human gut

shortbred_ardb_hmp_t2d.tsv

| Sample.ID | HMP1 | HMP2 | HMP3 | HMP4 | HMP5 | HMP6 | HMP7 | HMP8 | HMP9 | HMP10 | HMP11 | HMP12 | HMP13 | HMP14 | HMP15 | HMP16 | HMP17 | HMP18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dataset | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP | HMP |
| Gender | Female | Male | Female | Male | Female | Female | Male | Male | Female | Female | Female | Male | Male | Male | Female | Male | Male | Male |
| ABR Class | SRS011061 | SRS011134 | SRS011239 | SRS011271 | SRS011302 | SRS011405 | SRS011452 | SRS011529 | SRS011586 | SRS012273 | SRS012902 | SRS013158 | SRS013215 | SRS013476 | SRS013521 | SRS013687 | SRS013800 | SRS013951 |
| ABC Antibiot | 0 | 0.6097114 | 0.53837173 | 0 | 0 | 0.05083452 | 0 | 0 | 18.879238 | 0.3999418 | 0.6375002 | 0.11029351 | 0 | 0 | 0.1499069 | 3.3238466 | 0 | 0 |
| Aminoglycos | 0 | 0 | 0 | 0.5570841 | 0 | 0 | 0 | 0 | 0 | 0.4844142 | 0 | 0 | 0 | 7.15621993 | 0 | 0 | 0 | 0.06597383 |
| Aminoglycos | 11.8847826 | 2.3493412 | 1.31127279 | 2.1879248 | 1.70197254 | 25.2342538 | 0 | 1.4888313 | 6.7524558 | 11.6664297 | 0.2944691 | 0 | 0.54364476 | 22.1364669 | 1.0549423 | 6.1159491 | 2.1534126 | 2.95684284 |
| Aminoglycos | 0.72342527 | 9.510191 | 0.43478001 | 9.31863091 | 1.44994258 | 21.7649766 | 0 | 0 | 1.8219867 | 1.9941331 | 0.7220629 | 1.82419711 | 0 | 1.09356043 | 1.6969943 | 5.382002 | 1.6022915 | 0.98286613 |
| Antibiotic Ta | 0 | 0.4319648 | 0 | 0 | 0.11002037 | 0 | 0 | 0 | 0.1044046 | 0 | 0.6096981 | 4.45863298 | 0 | 0 | 0.1242086 | 0 | 0 | 0 |
| Chloramphe | 0 | 0.8931758 | 0.50566409 | 0.06863132 | 0 | 0 | 0 | 0 | 0.2300411 | 0.2286945 | 0 | 0 | 0 | 0 | 0 | 0 | 0.3360012 | 0 |
| Chloramphe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chloramphe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Class A Beta- | 11.9616538 | 14.1741569 | 192.732027 | 57.3421171 | 30.3784485 | 36.4756423 | 41.445191 | 77.8068337 | 27.5978829 | 84.7152993 | 29.5138602 | 4.47890136 | 7.54656865 | 6.17723545 | 67.6346059 | 121.5429 | 40.9881448 | 18.254292 |
| Class B Beta- | 0.73757867 | 0.4730655 | 0 | 0.35938332 | 0.22651252 | 0.45452038 | 0 | 0.1196987 | 1.5652141 | 0.5770399 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Class C Beta- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.4758603 | 0.2556631 | 0 | 0 | 0 | 0 | 0.1458178 | 0 | 0 |
| Class D Beta- | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Gene Modul | 0 | 0 | 0.12940327 | 0 | 0 | 0 | 0 | 0 | 0 | 2.6860575 | 0.3513343 | 0.52138395 | 0.18121492 | 0.09719297 | 0 | 0.6224941 | 0 | 0 |
| Gene Modul | 0 | 0 | 0.53609928 | 0.10341706 | 0.28813026 | 0 | 0 | 0.1033344 | 0 | 0.4529638 | 0 | 0.59939377 | 0 | 0.73268549 | 0 | 0 | 0 | 0.15287079 |
| Glycopeptide | 0 | 0.1148873 | 0.10721986 | 2.91192901 | 11.8252927 | 1.06129011 | 0 | 1.475885 | 0 | 3.8329823 | 0.2028631 | 0.17855513 | 0 | 2.57636295 | 0 | 12.8763448 | 0 | 1.37583708 |
| Lincosamide | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Macrolide Re | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.2216556 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MATE Antibi | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| MFS Antibiot | 0 | 0.1079916 | 2.44436309 | 2.24124166 | 0.15717195 | 19.6482667 | 0 | 0 | 0 | 6.0081483 | 4.73637 | 0.16432993 | 0 | 9.88061341 | 0.2382082 | 43.436675 | 1.4549685 | 0 |
| Other ARG | 0 | 0.1641248 | 1.50507872 | 4.90492355 | 0.80462657 | 0.27160156 | 0 | 0.4618416 | 1.2797248 | 2.911427 | 1.0099704 | 0.79420864 | 0 | 0.21818147 | 0.3167416 | 0.7025792 | 0 | 4.57893981 |
| Puromycin R | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Quinolone R | 0 | 0 | 0.05601037 | 0.09933481 | 0.05066727 | 0.05083452 | 0 | 0 | 0 | 0.8647162 | 0.1335553 | 3.29844229 | 0.06626516 | 0.6266389 | 0 | 0.1841579 | 0.1746919 | 0 |
| Rifamycin Re | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| RND Antibiot | 1.11005589 | 0.2116346 | 0.87820136 | 0.51112275 | 1.80007009 | 12.407319 | 34.237278 | 3.5262745 | 38.781576 | 4.5900824 | 1.9670192 | 0.17668244 | 38.004141 | 1.38795841 | 0.7786209 | 2.9700758 | 1.1984926 | 6.61769588 |
| rRNA Methyl | 5.61799582 | 6.0194576 | 37.2369165 | 9.44289101 | 34.6172522 | 94.7288439 | 2.051664 | 80.7900949 | 122.947846 | 2.4135554 | 10.2418695 | 0.06217665 | 7.23364421 | 13.9417838 | 130.737494 | 96.9503344 | 18.8879339 | 5.07069194 |
| SMR Antibiot | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.876332 | 0 | 0.08288129 | 0 | 0.19222828 | 0 | 0.2560272 | 0 | 0 |
| Streptogram | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Tetracycline | 0.06843748 | 2.6183624 | 0.57325559 | 0.86505449 | 12.8908188 | 0.16675423 | 2.793598 | 0.359161 | 0.5939219 | 2.0434753 | 2.4886453 | 0.33754257 | 0.23247387 | 0 | 0.9097696 | 2.3449461 | 0 | 5.81292995 |

# What it means: LEfSe

- Visit LEfSe at: http://huttenhower.sph.harvard.edu/galaxy/

Galaxy / Huttenhower Lab | Analyze Data | Workflow | Shared Data | Visualization | Help | User | Using 0%

Tools

search tools

HUTTENHOWER LAB MODULES

LEfSe

A) Format Data for LEfSe

B) LDA Effect Size (LEfSe)

C) Plot LEfSe Results

D) Plot Cladogram

E) Plot One Feature

F) Plot Differential Features

MetaPhlAn

GraPhlAn

microPITA

MaAsLin

PICRUSt

LOAD DATA MODULE

Get Data

Upload File from your computer

First click here

Thanks for visiting our lab's tools and applications page, implemented within the Galaxy web application and workflow framework. Here, we provide a number of resources for metagenomic and functional genomic analyses, intended for research and academic use. Please see the menus and folders to the left for an overview of available tools including documentation, sample data, and publications.

Our lab's research interests include metagenomics and the human microbiome, the relationships between microbial communities and human health, microbiome systems biology, and large-scale computational methods for studying all of these areas. In addition to the tools provided here, feel free to take a look at our additional research and publications, including the Sleipnir library for computational functional genomics.

The tools are available here without account creation. However, you are strongly invited to create an account for having access to the history, saved analyses, datasets and workflows. You can create an account and/or log in using the User menu in the top-right corner.

If you have any comments, questions, or suggestions, please contact Dr. Huttenhower.

History

Unnamed history

0 bytes

This history is empty. You can load your own data or get data from an external source

# What it means: LEfSe

- Then upload your formatted table
  - After you upload, wait for the progress meter to turn green!

Galaxy / Huttenhower Lab | Analyze Data | Workflow | Shared Data | Visualization | Help | User | Using 0%

Tools

search tools

HUTTENHOWER LAB MODULES

LEfSe

A) Format Data for LEfSe

B) LDA Effect Size (LEfSe)

C) Plot LEfSe Results

D) Plot Cladogram

E) Plot One Feature

F) Plot Differential Features

MetaPhlAn

GraPhlAn

microPITA

MaAsLin

PICRUSt

LOAD DATA MODULE

Get Data

Upload File from your computer

DEFAULT GALAXY MODULES

Upload File (version 1.1.4)

File Format:

Auto-detect

Which format? See help below

1. Click here, browse to **shortbred_ardb_hmp_t2d.tsv**

Choose File

.ab.filtered.metadata.txt

browser limitations, uploading files larger than 2GB is guaranteed to fail. To upload large files, use the URL method (below) or FTP (if enabled by the site administrator).

URL/Text:

Here you may specify a list of URLs (one per line) or paste the contents of a file.

Convert spaces to tabs:

Yes

Use this option if you are entering intervals by hand.

Genome:

unspecified (?)

2. Then here

Execute

History

Unnamed history

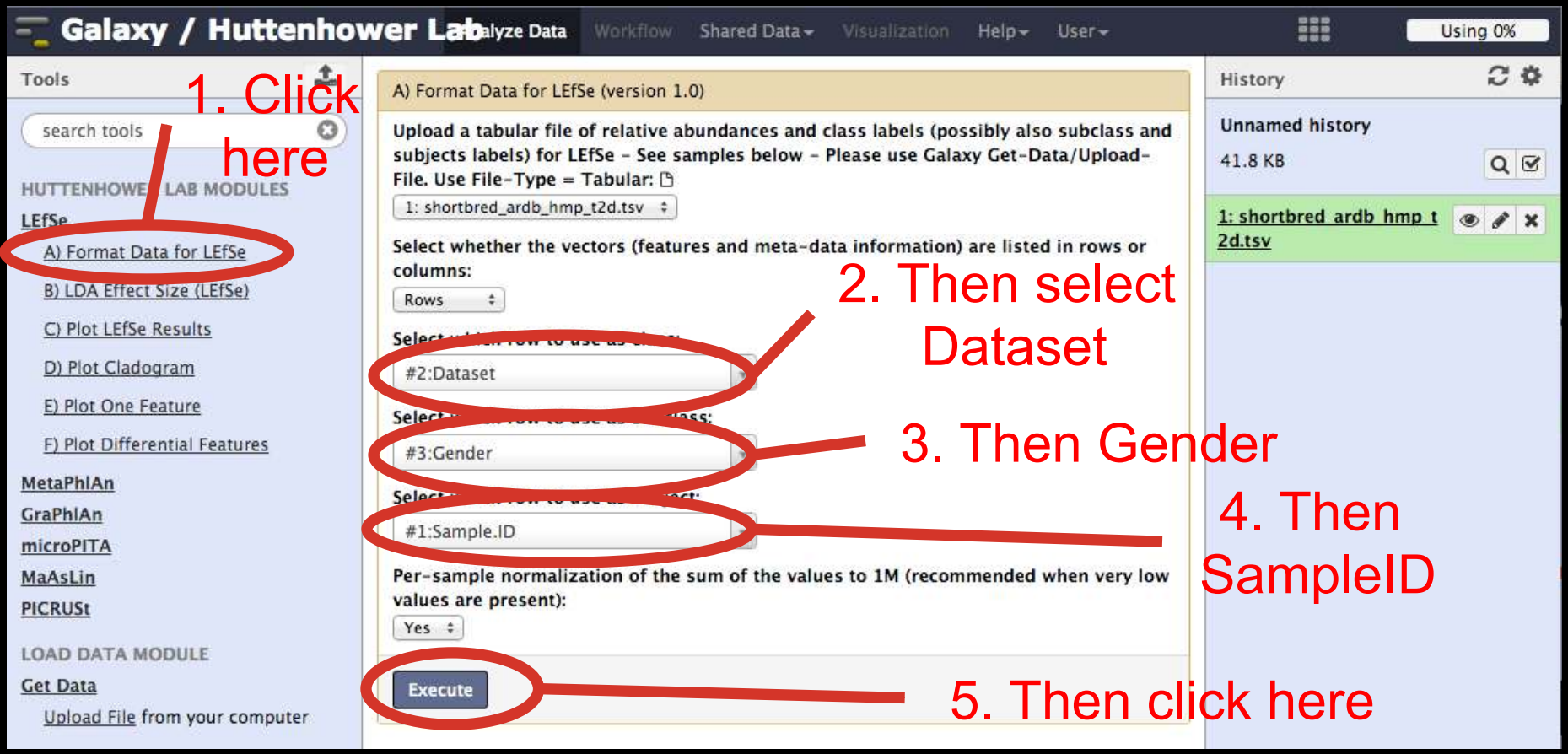
269.2 KB

This history is empty. You can load your own data or get data from an external source

3. Then watch here

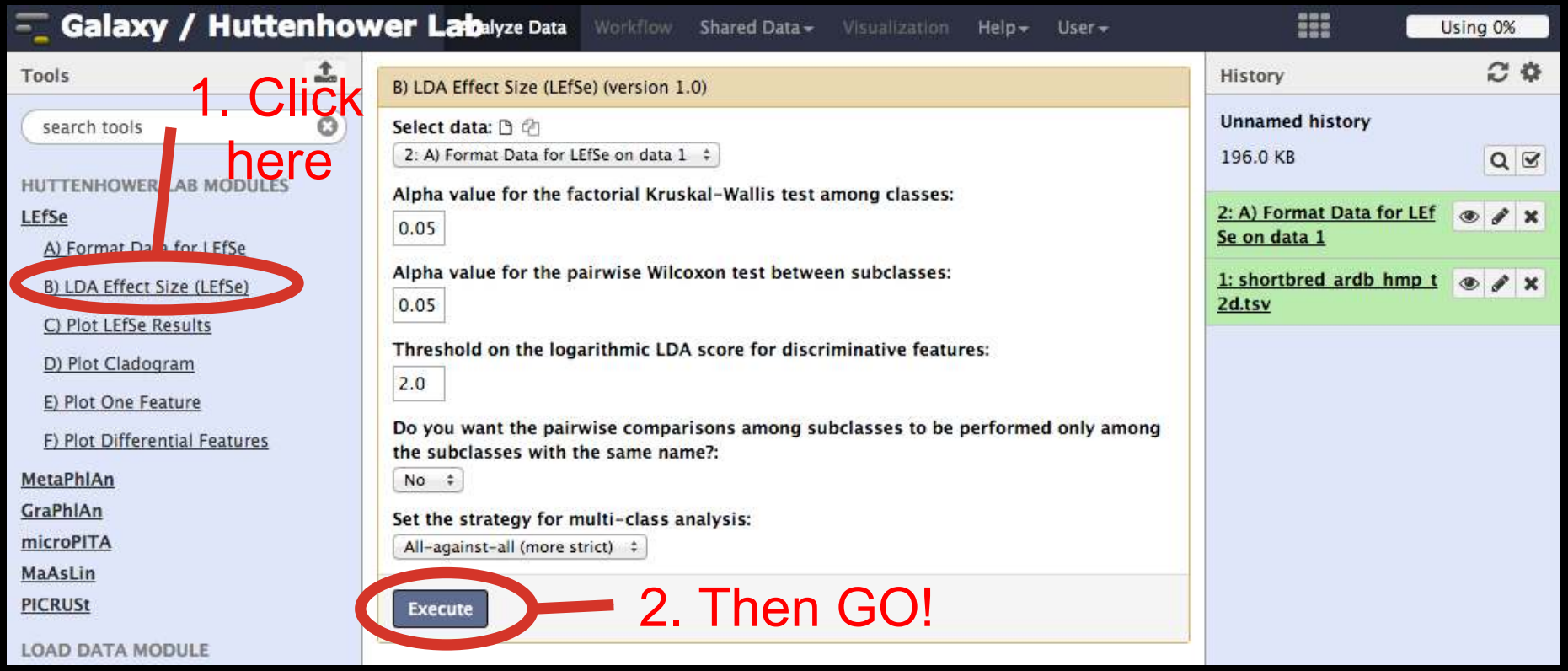
# What it means: LEfSe

- Then tell LEfSe about your metadata:



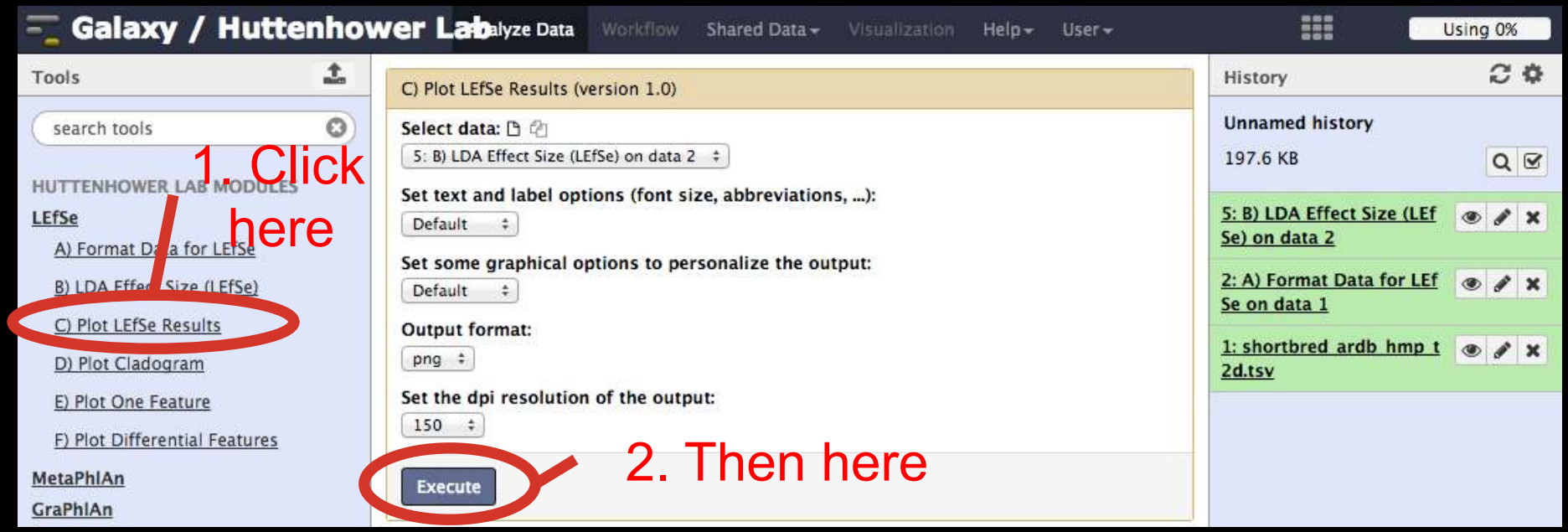
# What it means: LEfSe

- Leave all parameters on defaults, and run LEfSe!
  - You can try playing around with these parameters if desired



# What it means: LEfSe

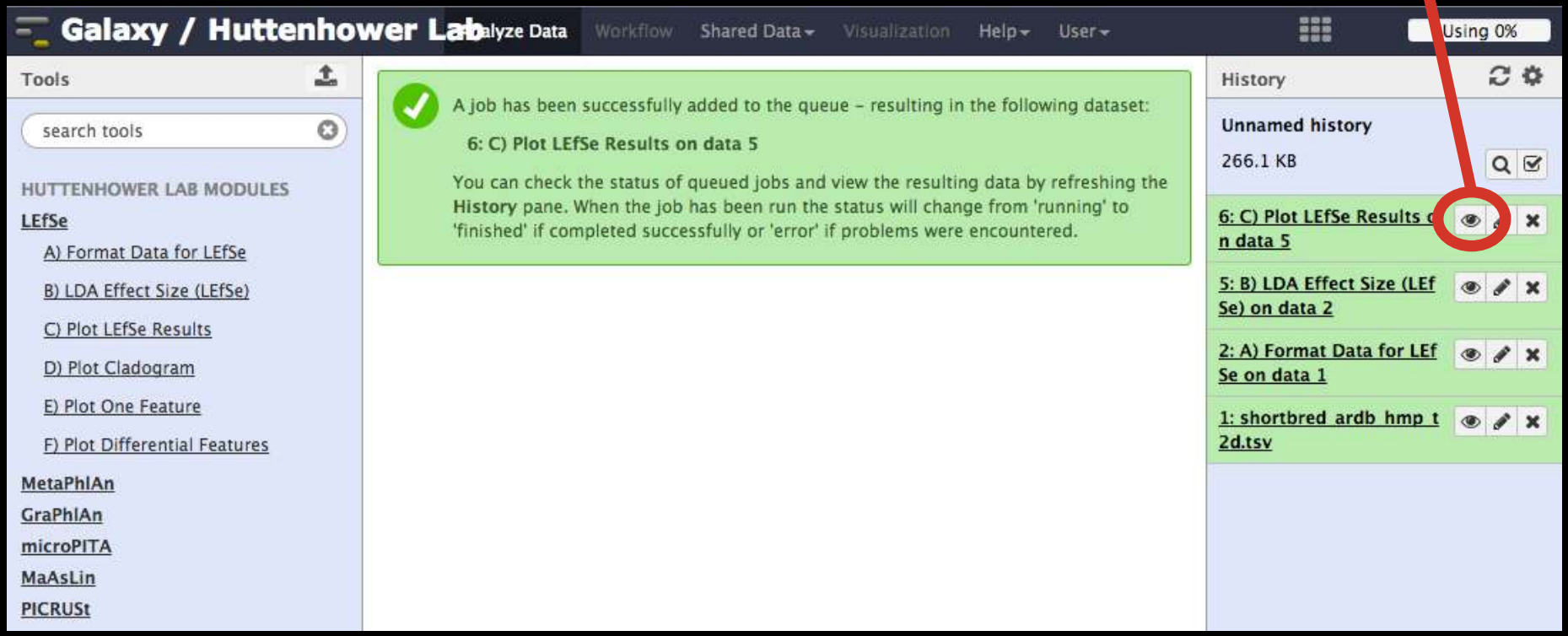
- You can plot the results as a bar plot
  - Again, lots of graphical parameters to modify if desired



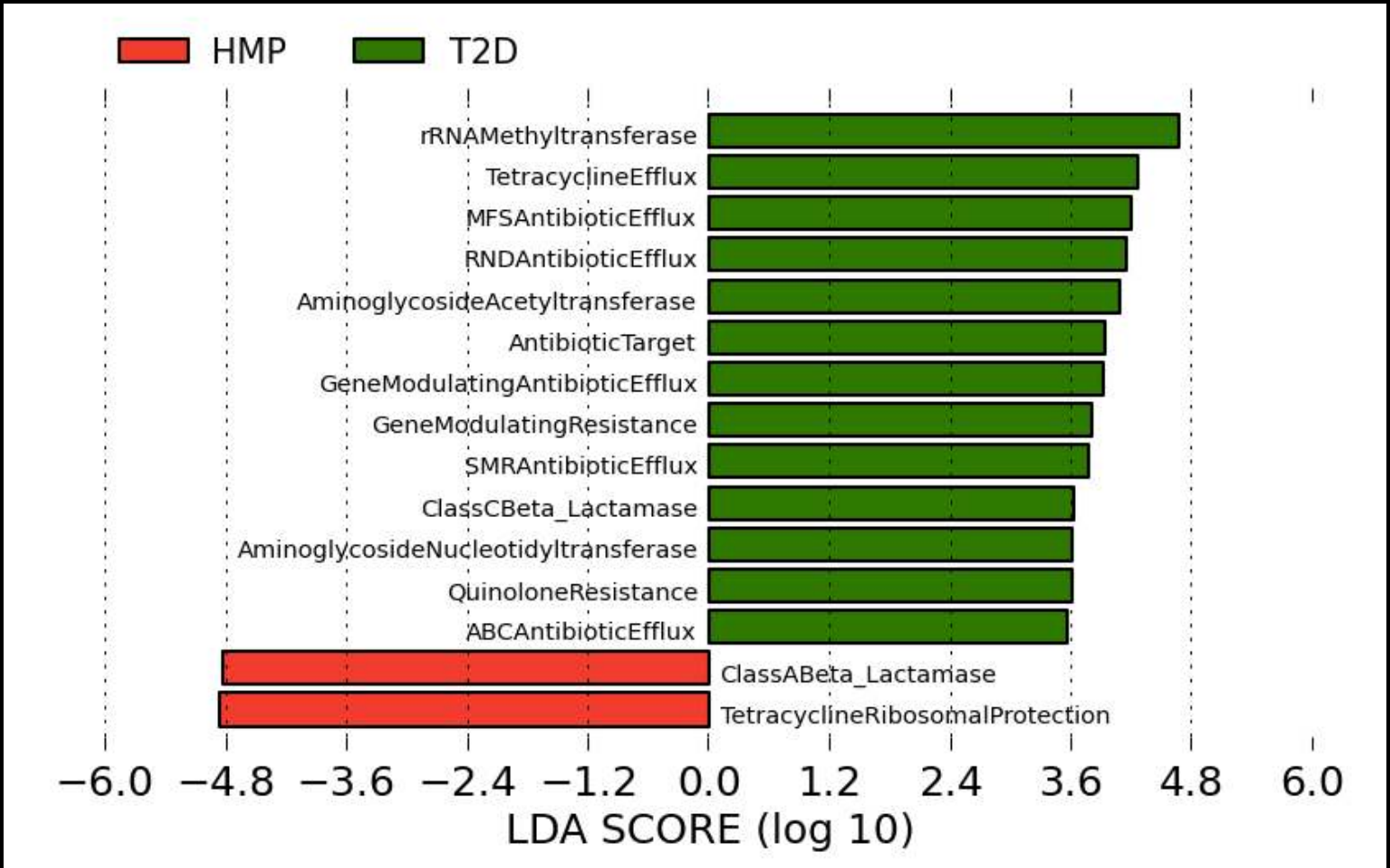
# What it means: LEfSe

- In Galaxy, view a result by clicking on its “eye”

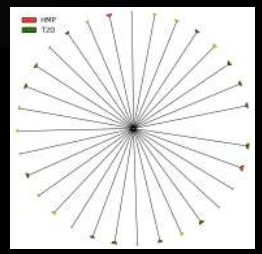
Click here



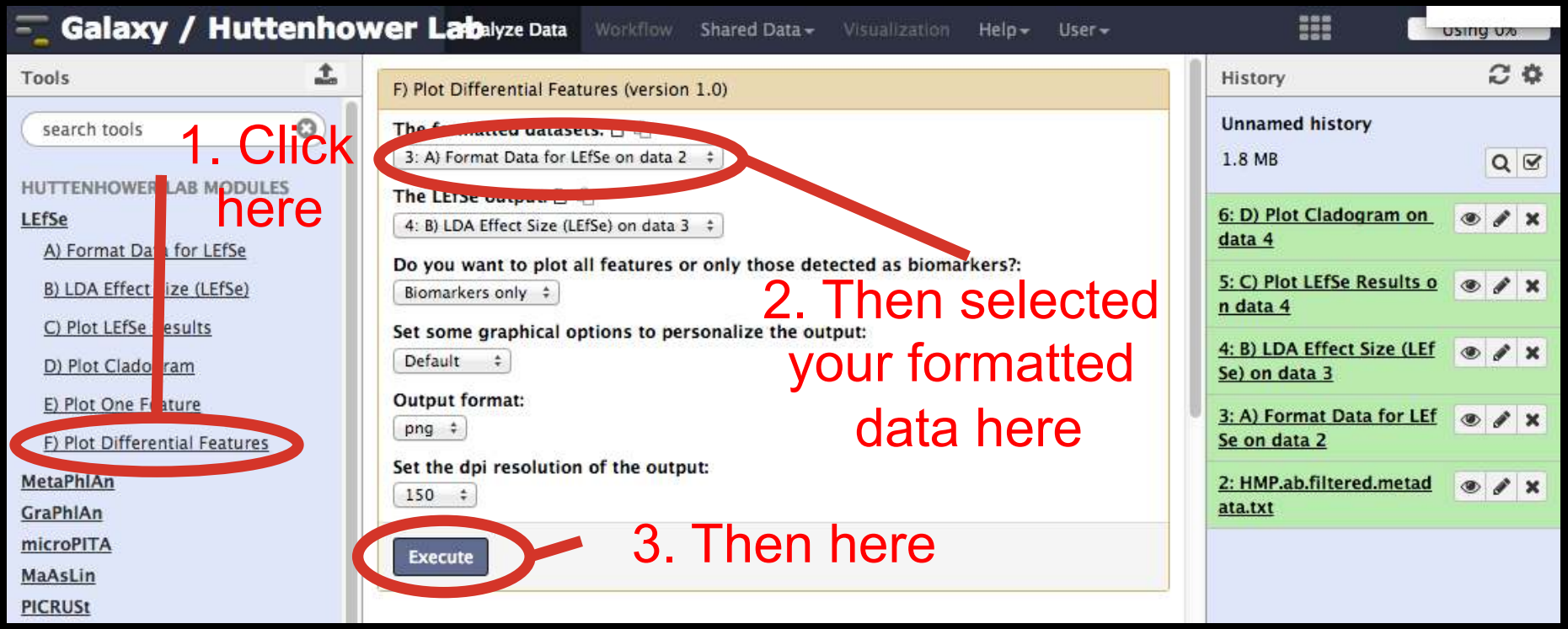
# What it means: LEfSe



# What it means: LEfSe



- There's no really any reason to plot a cladogram
  - Although it will work!
- But you can see the raw data for individual biomarkers
  - These are generated as a zip file of individual plots



# What it means: LEfSe

- In Galaxy, download a result by clicking on its “disk”

Click here

Galaxy / Huttenhower Lab Analyze Data Workflow Shared Data Visualization Help User Using 0%

Tools

search tools

HUTTENHOWER LAB MODULES

LEfSe

A) Format Data for LEfSe

B) LDA Effect Size (LEfSe)

C) Plot LEfSe Results

D) Plot Cladogram

E) Plot One Feature

F) Plot Differential Features

MetaPhlAn

GraPhlAn

microPITA

MaAsLin

PICRUSt

LOAD DATA MODULE

Get Data

A job has been successfully added to the queue – resulting in the following dataset:

8: F) Plot Differential Features on data 2 and data 5

You can check the status of queued jobs and view the resulting data by refreshing the **History** pane. When the job has been run the status will change from 'running' to 'finished' if completed successfully or 'error' if problems were encountered.

History

Unnamed history

1020.9 KB

8: F) Plot Differential Features on data 2 and data

2,363 lines

format: **zip**, database: ?

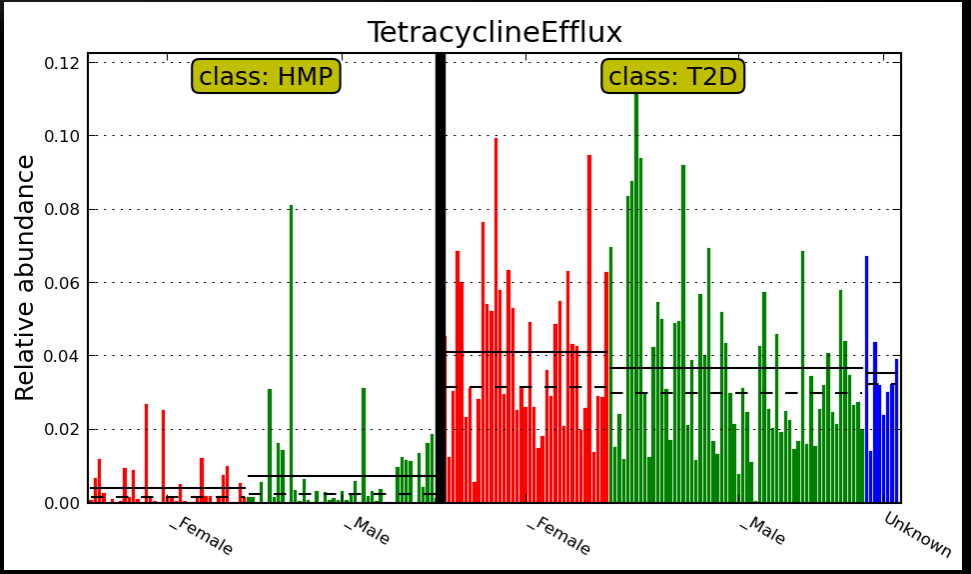
Exporting MFSAntibioticEfflux
Exporting ClassCBeta_Lactamase
Exporting AminoglycosideAcetyltransferase
Exporting rRNAMethyltransferase
Exporting ClassABeta_Lactamase
Exporting AntibioticTarget
Exporting TetracyclineRibosomalProtection
Exporting G

binary file

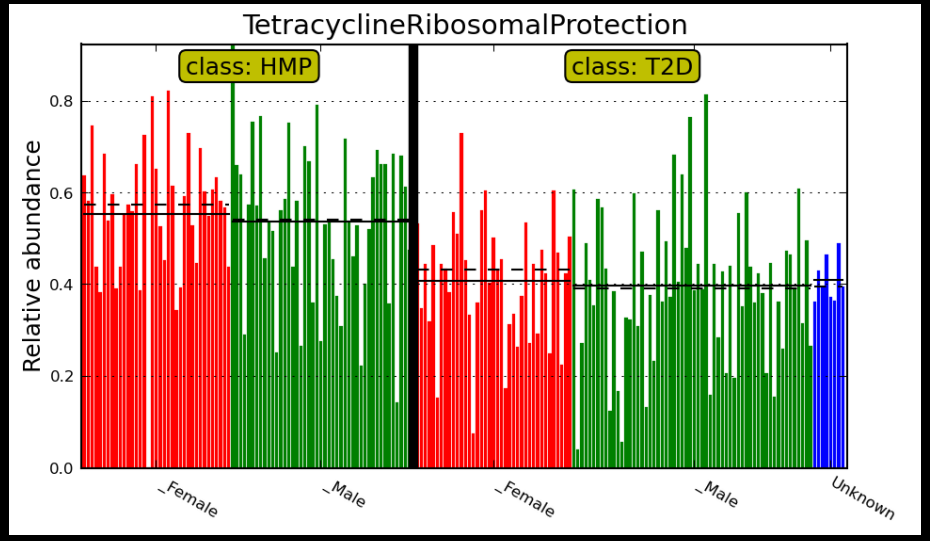
Then click here

# What it means: LEfSe

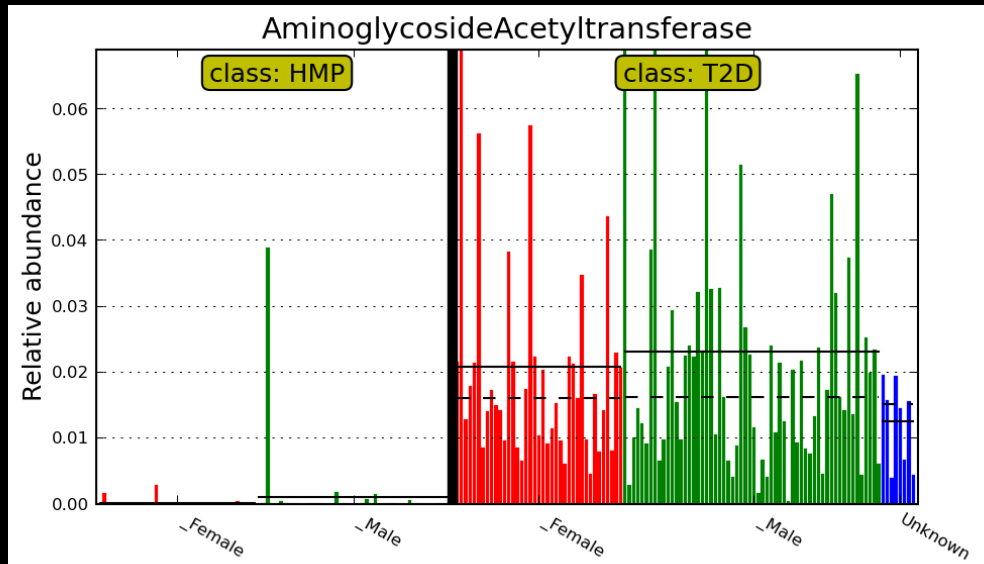
Tetracycline Efflux Pumps




Tet. Ribosomal Blockers




Aminoglycoside Acetyltransferases



# Summary

- HUMAnN
  - Quality-controlled metagenomic reads in
  - Tab-delimited gene, module, and pathway relative abundances out

- ShortBRED
  - Raw metagenomic reads,
    Proteins of interest, and
    Protein reference database in
  - Tab-delimited gene family rel. abundances out

http://huttenhower.sph.harvard.edu/biobakery

# Huttenhower Lab Tools

Welcome to the official Huttenhower Tutorials wiki.

**We now support `bioBakery`, a virtual environment platform that provides Huttenhower tools (already installed!). Please click on the button below for more information:**

The wiki provides tutorials for Huttenhower tools, illustrating through demos how to use these tools on your datasets. Huttenhower tools can be divided under three main categories as shown below. Click on the tool for the corresponding tutorial.

## Composition Analysis

These tools can determine the composition in terms of (i) microbial species and their associated abundances (MetaPhlAn) or (ii) genes and associated pathways (HUMAnN) in the dataset. Please click on the links below for detailed tutorials:

## Statistical Analysis

These tools can determine the associations from the provided metadata information and microbial composition tables. Please click on the links below for detailed tutorials:

## Visualization

These tools can help visualize taxonomical and phylogenetic information for (i) microbial composition/taxonomy data, (ii) outputs from MetaPhlAn, LEfSe, HUMAnN, MaAsLin. Please click on the link below for detailed tutorial:

# Thanks!

http://huttenhower.sph.harvard.edu

Alex Kostic

Levi Waldron

Xochitl Morgan

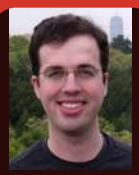
Tim Tickle

Daniela Boernigen

Lauren McIver

George Weingart

Emma Schwager


Eric Franzosa

Boyu Ren

Tiffany Hsu

Ali Rahnavard

Ayshwarya Subramanian

Jim Kaminski

Regina Joice

Koji Yasuda

Kevin Oh

Galeb Abu-Ali

Afrah Shafquat

Randall Schwager

Chengwei Luo

Keith Bayer

Moran Yassour

Alexandra Sirota

Dirk Gevers

Ramnik Xavier

Wendy Garrett

Andy Chan

## Human Microbiome Project 2

Lita Procter
Jon Braun
Dermot McGovern
Subra Kugathasan
Ted Denson
Janet Jansson
Bruce Birren
Chad Nusbaum
Clary Clish
Joe Petrosino
Thad Stappenbeck

BROAD INSTITUTE

## Human Microbiome Project

Jane Peterson
Sarah Highlander
Barbara Methe
Karen Nelson
George Weinstock
Owen White

Nicola Segata

Gautam Dantas
Molly Gibson

Katherine Lemon

Brendan Bohannan
James Meadow