Metagenomic taxonomic profiling with MetaPhlAn2

Galeb Abu-Ali
Eric Franzosa
Curtis Huttenhower

09-17-15

Harvard T.H. Chan School of Public Health
Department of Biostatistics

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:

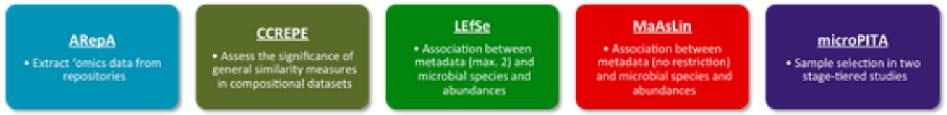
The wiki provides tutorials for Huttenhower tools, illustrating through demos how to use these tools on your datasets. Huttenhower tools can be divided under three main categories as shown below. Click on the tool for the corresponding tutorial.

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:

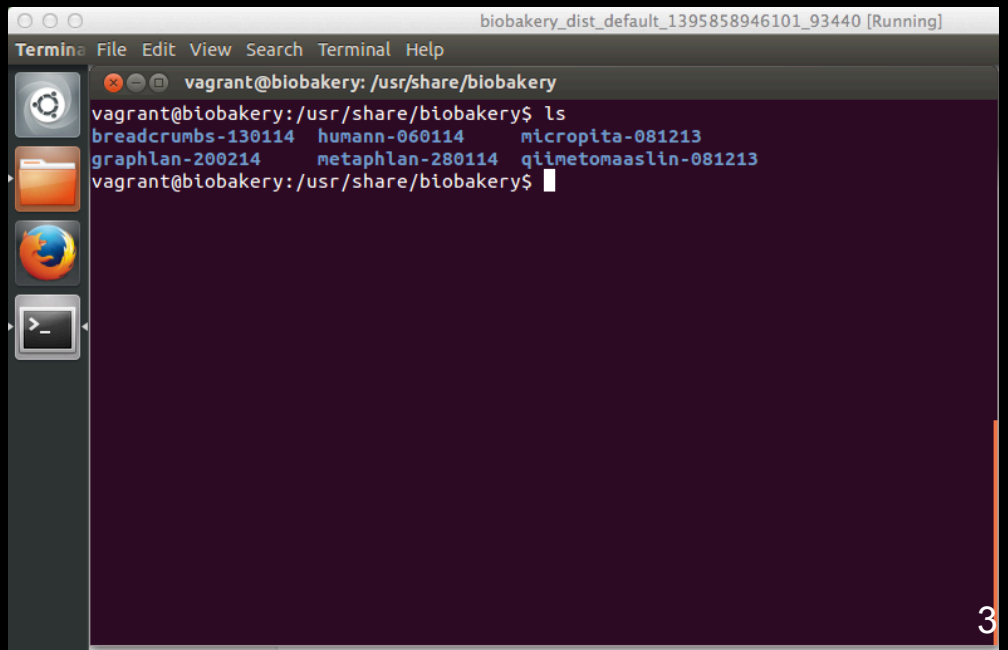


Visualization

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:

The bioBakery: a next-generation environment for microbiome analyses

- Environment for meta'ome analysis
  - Shotgun metagenomes/transcriptomes
  - Taxonomic and functional profiling
  - Experimental design, statistical analysis
- Pre-built one-click environments to run:
  - On your laptop graphically
  - On a server remotely
  - On the cloud (Amazon)



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

The two big questions…

Who is there?

(taxonomic profiling)

What are they doing?

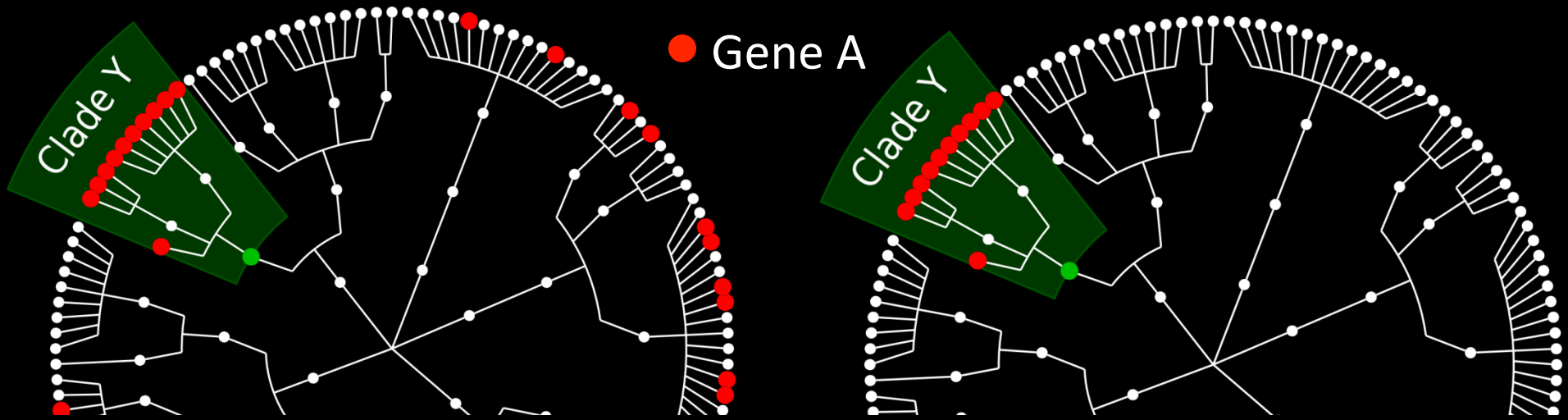
(functional profiling)

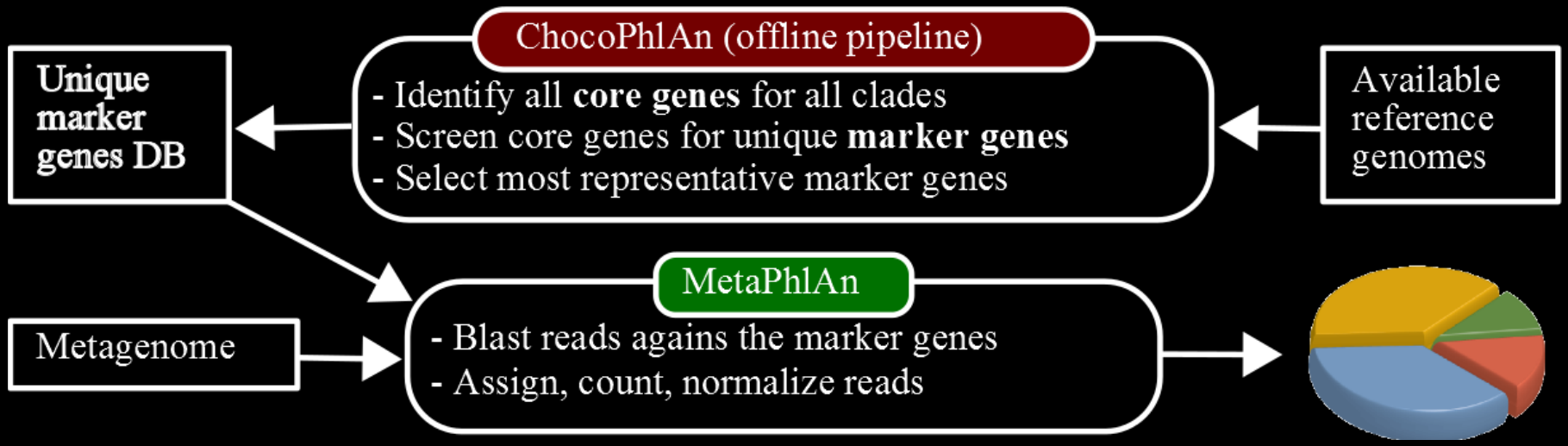
MetaPhlAn overview

Nicola Segata

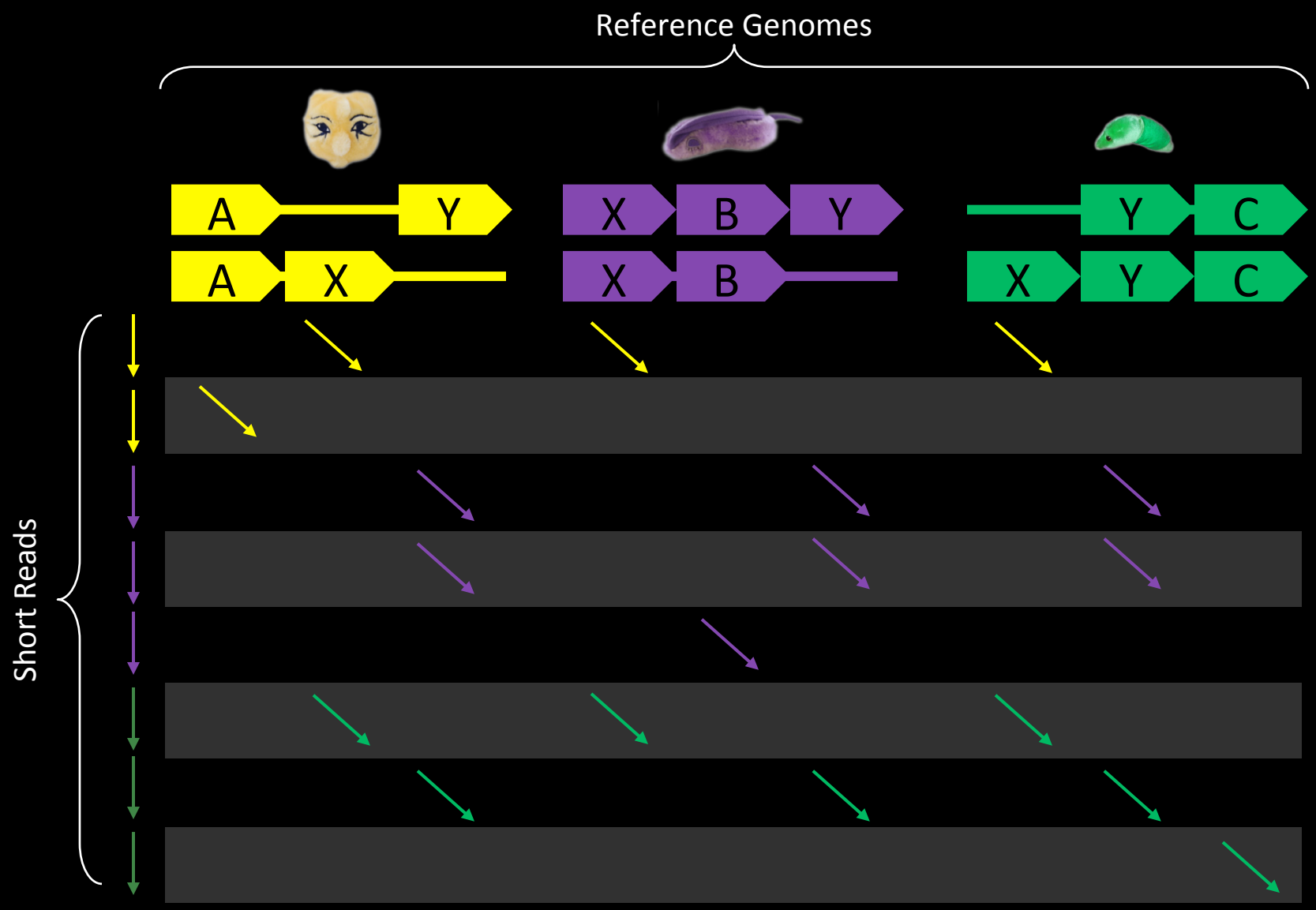
A is a core gene for clade Y

A is a unique marker gene for clade Y

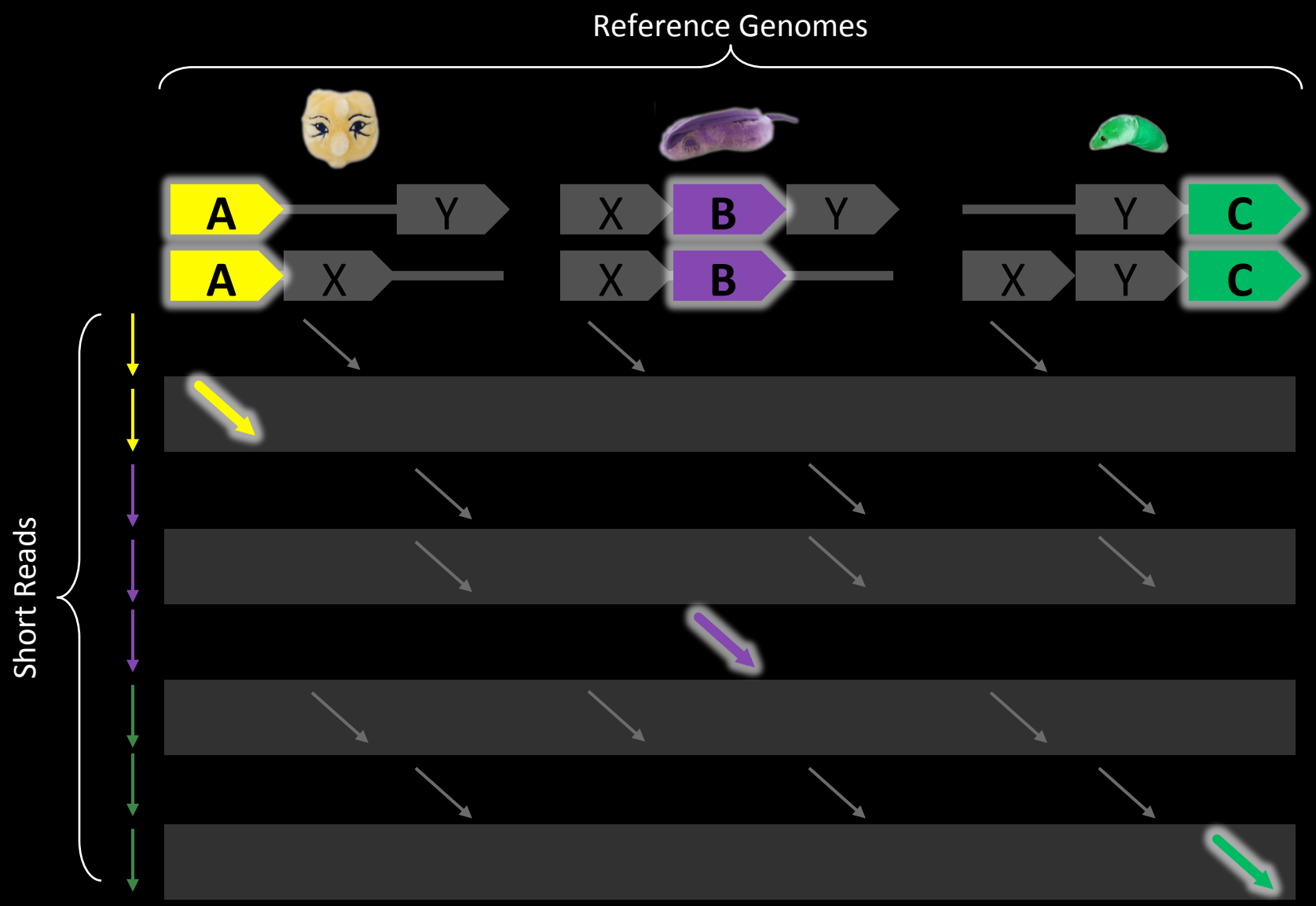




MetaPhlAn
Metagenomic Phylogenic Analysis

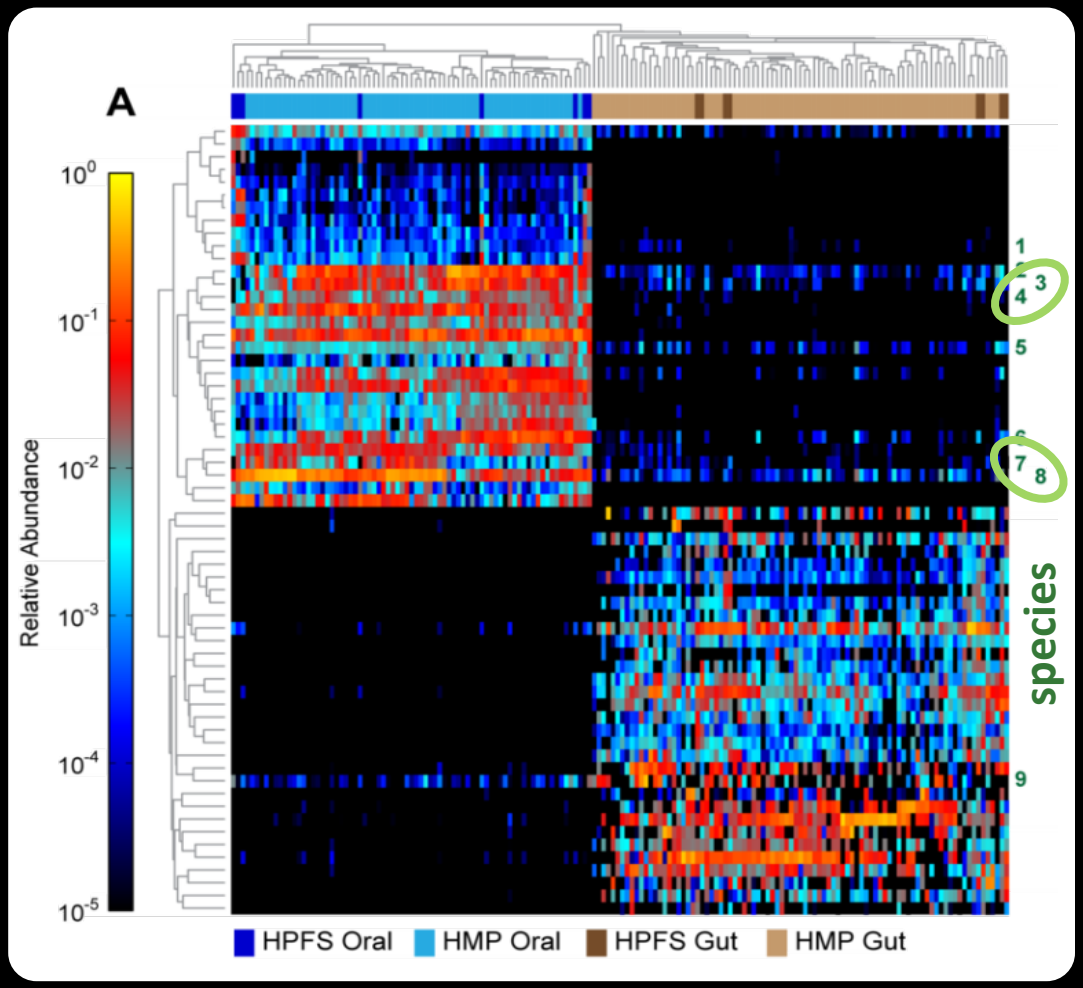


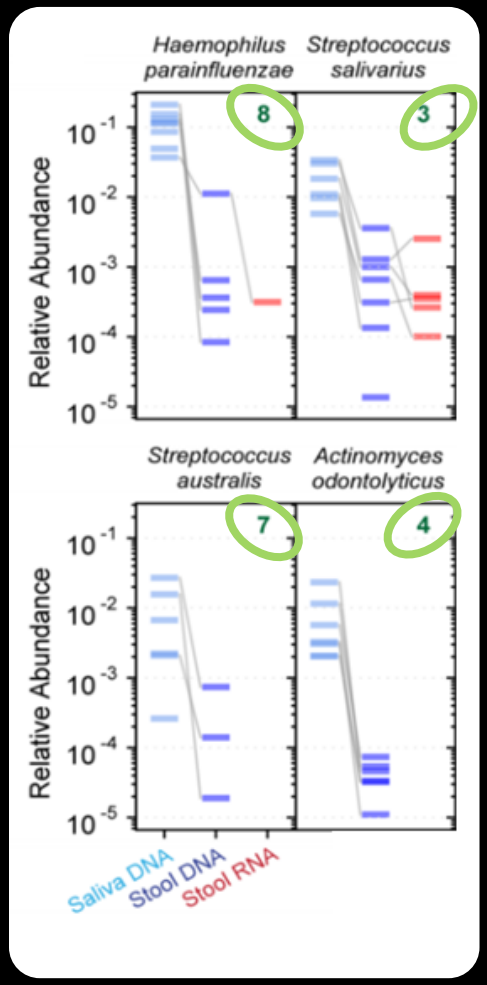
MetaPhlAn
Metagenomic Phylogenic Analysis



MetaPhlAn in action

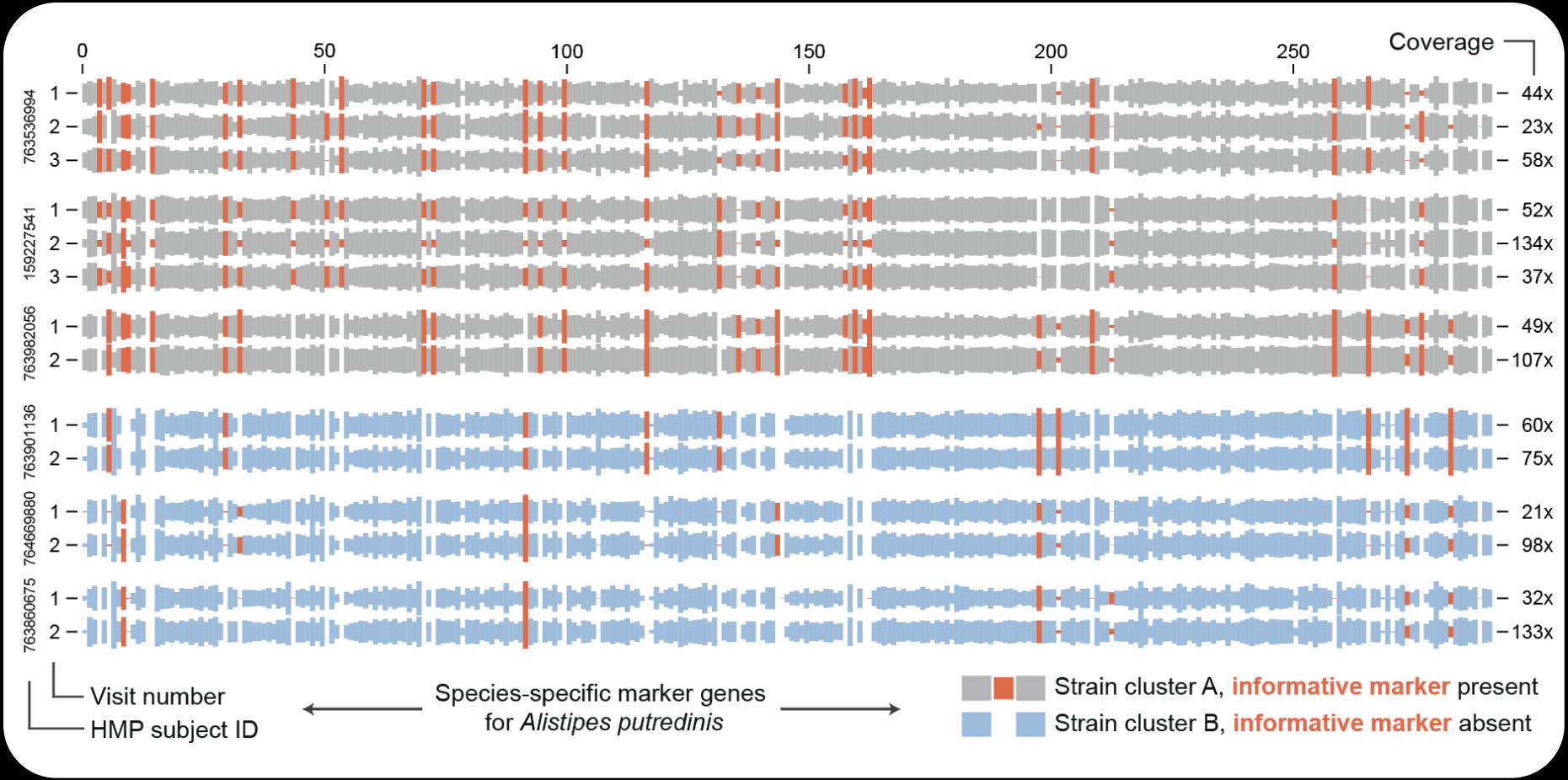
Eric Franzosa





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

MetaPhlAn in action: *strain profiling*



- In practice, not all markers are present
- Individual-specific marker “barcodes”
- Often very stable over time

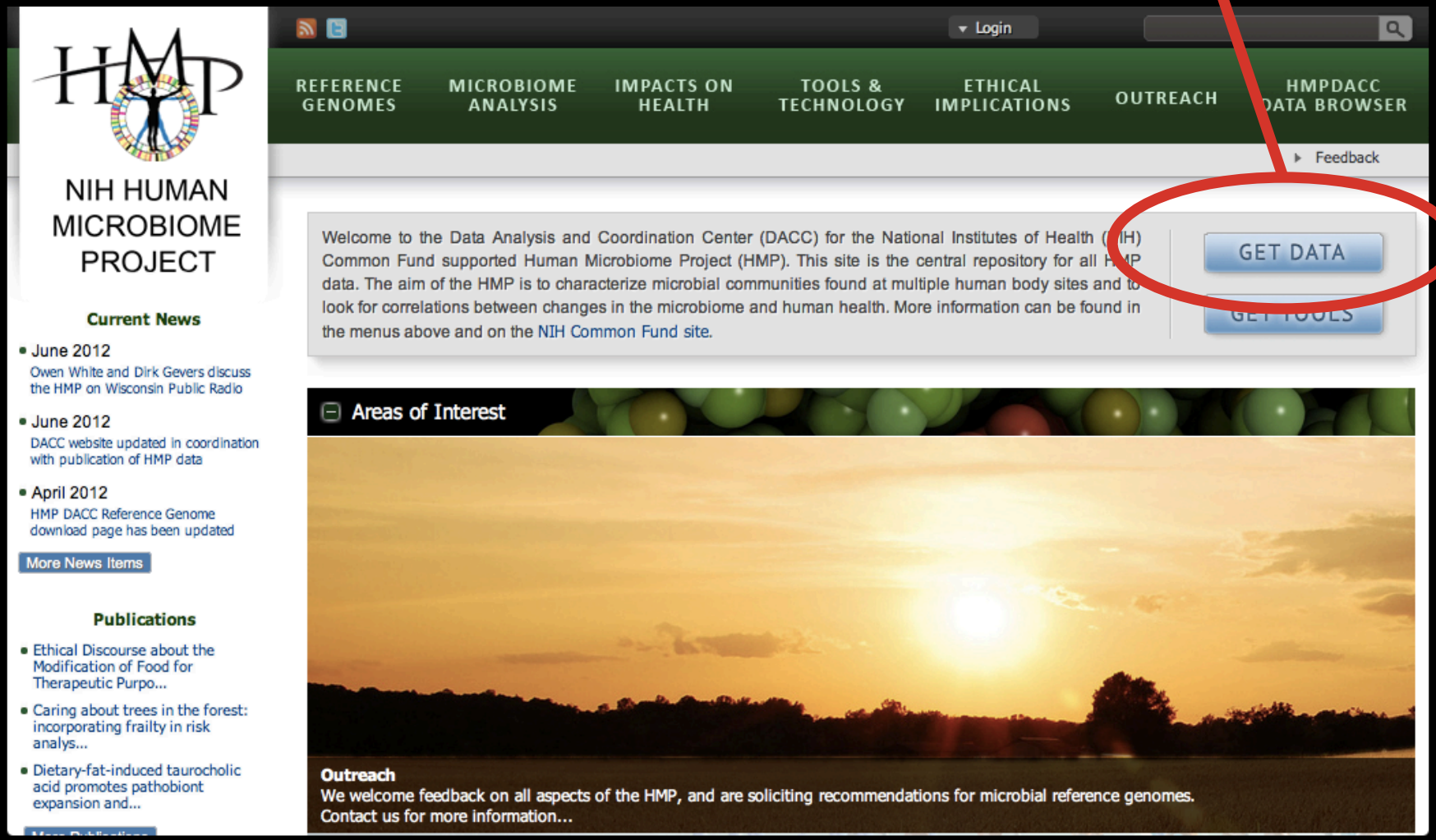
Some setup notes

- 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`**.

Getting some HMP data

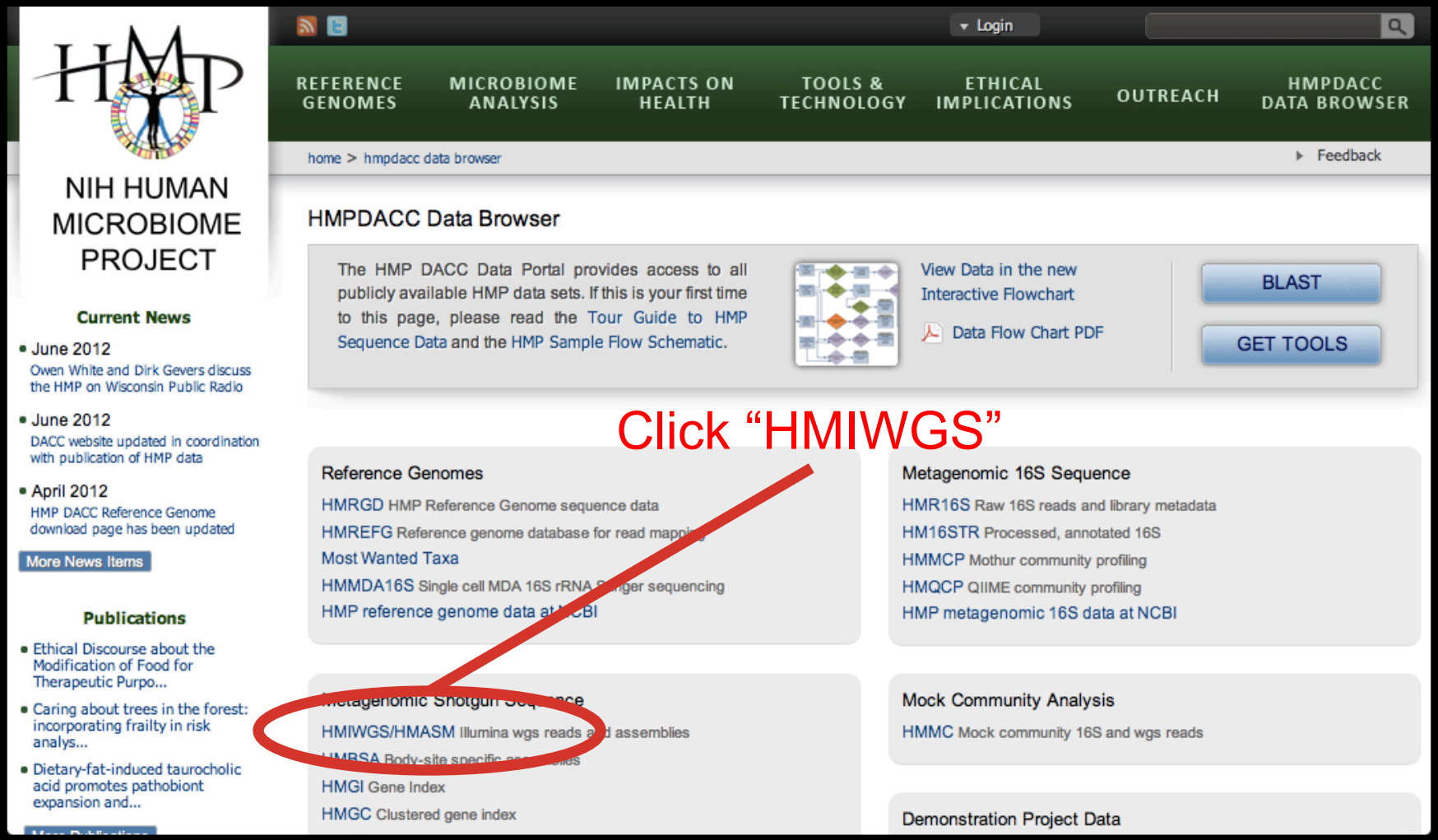
- Go to http://hmpdacc.org

Click "Get Data"



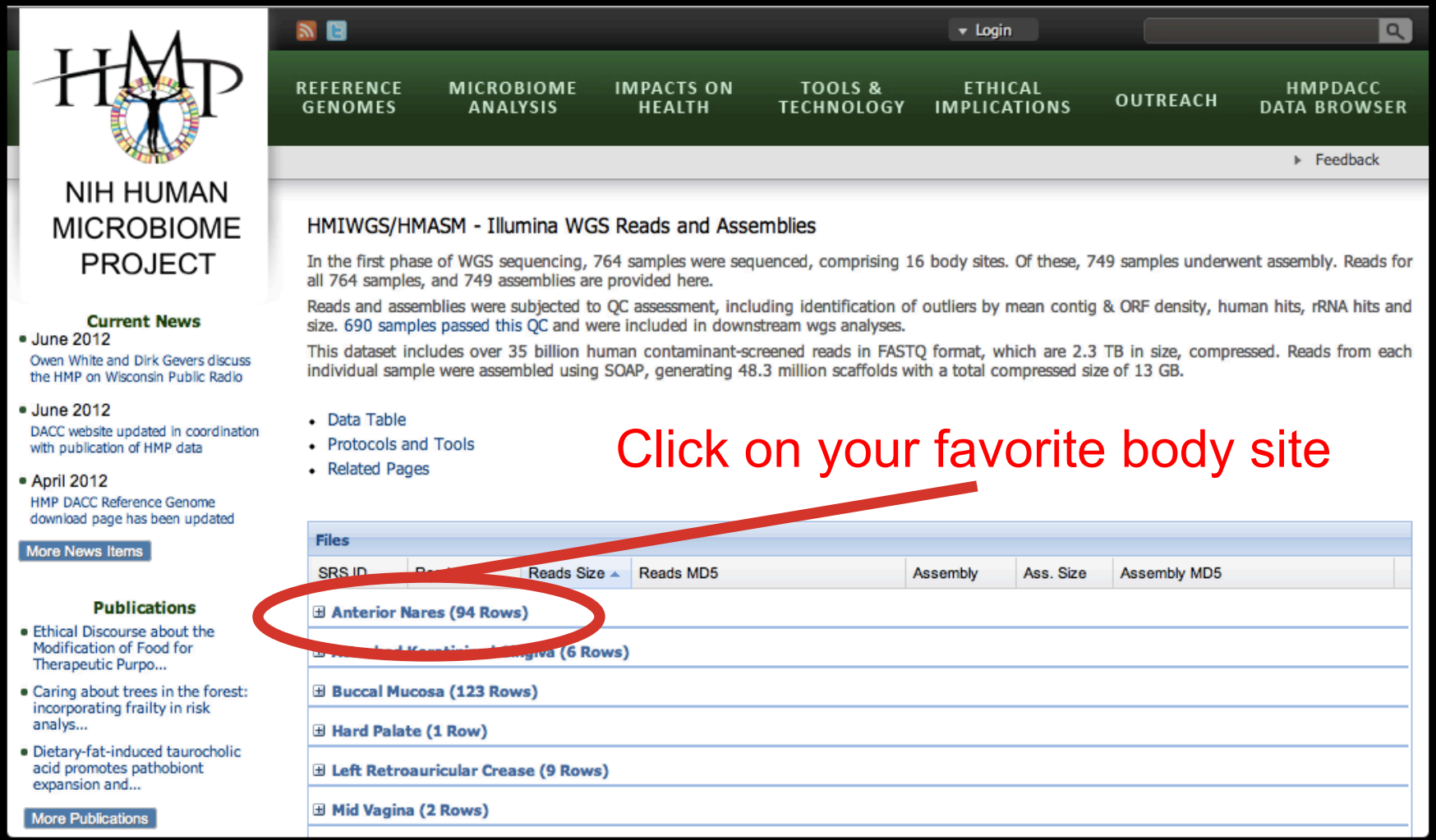
Getting some HMP data

- Check out what's available



Getting some HMP data

- Check out what's available



Getting some HMP data

Don’t click on anything!

- Check out what’s available

April 2012

HMP DACC Reference Genome download page has been updated

More News Items

Publications

- Ethical Discourse about the Modification of Food for Therapeutic Purpo...
- Caring about trees in the forest: incorporating frailty in risk analys...
- Dietary-fat-induced taurocholic acid promotes pathobiont expansion and...

More Publications

Data Resources

- Tools & Protocols
- BLAST against Reference Genomes
- Project Catalog
- Access to Strains
- Clinical Sampling
- Most Wanted Resource

Files

SRS ID	Reads	Reads Size	Reads MD5	Assembly	Ass. Size	Assembly MD5
Anterior Nares (94 Rows)						
SRS047708	FTP	1.7 MB	d786590ff7fec20e8967127991766029	FTP	1.3 KB	ed98eda02d80a137c52b6fa8a3c57833
SRS019215	FTP	10.1 MB	55de248bbfa8c1bbf4447d007330f7ff	FTP	12.1 KB	cab8918433280eafc3d8f6ad78dc1ff7
SRS063178	FTP	13.1 MB	336f0b31b92880224c91ad52c4784adc	FTP	10.7 KB	99de257f1942e98bf1c052e2d046df33
SRS065179	FTP	13.3 MB	27b2c9209bc56cbe219d8c65fa32296c	FTP	54.6 KB	bb8b0d62a3c1923abfcaea01a598a60a
SRS065142	FTP	13.5 MB	3b05d6fcb205106fbd03f314e39f6d63	FTP	7.6 KB	91177065cf438056f2bfc67e99562fe4
SRS018585	FTP	16.8 MB	9d4129d2f5fdd51b9fc899bd84c47b5b	FTP	7.9 KB	aa9e9857b26b9efb4fa39bfaf101dc9d
SRS015640	FTP	17.6 MB	595baf36d8b3dcdd21149b3086ccbbee	FTP	52.4 KB	1c7a464db2fccce17c02f9600c867cb1
SRS056210	FTP	18.1 MB	9b2f74b8067e6f20551e6d3b48124c42	FTP	18.3 KB	c4abace0ec0b3e7e5ce1513cb8270e56
SRS018312	FTP	18.9 MB	2454e80d7e5216adf8d5b1850c98738c	FTP	25.4 KB	4f5f760eadd77782862669263e1b1d9d
SRS015450	FTP	18.9 MB	eefc0dcf2d52ca5251b01860d54d2bb5	FTP	107.1 KB	4e0a83868f2fb44f1788dfe1aaa5e13f
SRS049744	FTP	21.5 MB	6d9e2ffc82b08ef37551e902096e4c98	FTP	14.3 KB	da7a1cddd3c84b121ff49086432d25d3
SRS012291	FTP	21.9 MB	12775f5df6e71961f1c544e84f6c7342	FTP	8.9 KB	17b5110d391817c7ce52b7c1026df1ba
SRS051600	FTP	22.2 MB	391775b95926a221b8a3cde54a79ae22	FTP	13.9 KB	6db7007edd32b534bc918aad42d600ae
SRS019339	FTP	23.1 MB	76a621d6503d11d1a133a023dc240ae5	FTP	57.3 KB	9255d8206f10ac2611cf45270daa166c
SRS017244	FTP	23.5 MB	b7c2dec67738f317cb8826c09e1a9e39	FTP	21.3 KB	9bcf59e6b4fe15a4e8ccacb0bc824ba8
SRS018671	FTP	24.0 MB	7548b06b37038440c5420f7677ff7371	FTP	135.4 KB	4a180e3ea42a46bcea0a9441b137f243

Show All | Save As CSV File

Protocols and Tools

Getting some (prepped) HMP data

- Connect to the server instead
  - `cd` to your favorite directory and run:

```
for S in `ls /home/ubuntu/metagenomics/data/input/7*.fasta`;
do ln -s $S; done
```

- These are subsamples of six HMP files:
  - SRS014459.tar.bz2 ➔ 763577454-SRS014459-Stool.fasta
  - SRS014464.tar.bz2 ➔ 763577454-SRS014464-Anterior_nares.fasta
  - SRS014470.tar.bz2 ➔ 763577454-SRS014470-Tongue_dorsum.fasta
  - SRS014472.tar.bz2 ➔ 763577454-SRS014472-Buccal_mucosa.fasta
  - SRS014476.tar.bz2 ➔ 763577454-SRS014476-Supragingival_plaque.fasta
  - SRS014494.tar.bz2 ➔ 763577454-SRS014494-Posterior_fornix.fasta
- All six shotgunned body sites from
  - One subject, first visit
  - Subsampled to 20,000 reads

Who's there: MetaPhlAn1

- We won't use it today, but the first version of MetaPhlAn is at: http://huttenhower.sph.harvard.edu/metaphlan

The Huttenhower Lab

Department of Biostatistics, Harvard School of Public Health

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

Home

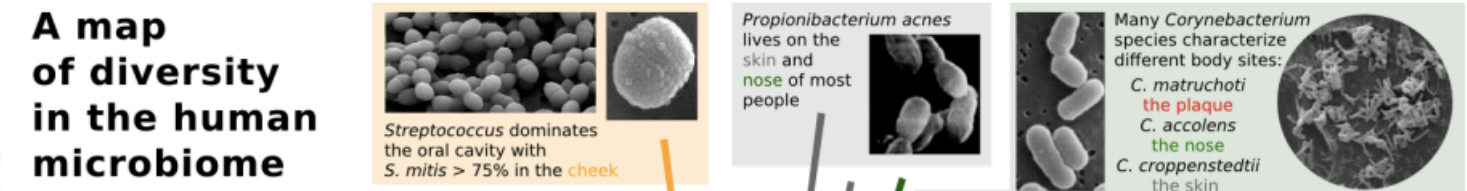
MetaPhlAn: Metagenomic Phylogenetic Analysis

MetaPhlAn is a computational tool for profiling the composition of microbial communities from metagenomic shotgun sequencing data. MetaPhlAn relies on unique clade-specific marker genes identified from 3,000 reference genomes, allowing:

- up to 25,000 reads-per-second (on one CPU) analysis speed (orders of magnitude faster compared to existing methods);
- unambiguous taxonomic assignments as the MetaPhlAn markers are clade-specific;
- accurate estimation of organismal relative abundance (in terms of number of cells rather than fraction of reads);
- species-level resolution for bacterial and archaeal organisms;
- extensive validation of the profiling accuracy on several synthetic datasets and on thousands of real metagenomes.

Please refer to the **MetaPhlAn paper** for additional information, validations, and examples. Also the **main paper of the Human Microbiome Project** uses MetaPhlAn (version 1.1) for species-level metagenomic profiling.

Here is an **infographic** of the application of the **Human Microbiome Project** results obtained applying MetaPhlAn on the 690 shotgun sequencing samples. Email **me** for a high-resolution version. This infographic also appears in a slightly modified version as the main illustration of a **New York Times article** by Carl Zimmer available **here** (NY Times subscription needed) and **here** (NY Times copyrighted version).



Who's there: MetaPhlAn2

- Instead, go to http://huttenhower.sph.harvard.edu/metaphlan2

The Huttenhower Lab
Department of Biostatistics, Harvard School of Public Health

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

Home

You *could* download MetaPhlAn2 by clicking here

MetaPhlAn v2.0

MetaPhlAn v2.0: Metagenomic Phylogenetic Analysis

MetaPhlAn is a computational tool for profiling the composition of microbial communities from metagenomic shotgun sequencing data. MetaPhlAn relies on unique clade-specific marker genes identified from ~17,000 reference genomes (~13,500 bacterial and archaeal, ~3,500 viral, and ~110 eukaryotic), allowing:

- up to 25,000 reads-per-second (on one CPU) analysis speed (orders of magnitude faster compared to existing methods);
- unambiguous taxonomic assignments as the MetaPhlAn markers are clade-specific;
- accurate estimation of organismal relative abundance (in terms of number of cells rather than fraction of reads);
- species-level resolution for bacteria, archaea, eukaryotes and viruses;
- extensive validation of the profiling accuracy on several synthetic datasets and on thousands of real metagenomes.

Obtaining MetaPhlAn v2.0

MetaPhlAn v2.0 can be obtained via the **MetaPhlAn v2.0 Bitbucket repository.**
The repository contains the source code and database files used to run MetaPhlAn v2.0, as well as a README file that includes the following information:

- Downloading MetaPhlAn v2.0
- Installation
- Detailed instruction on running MetaPhlAn v2.0

Tutorials

MetaPhlAn v2.0 tutorial is also available here. The tutorial contains a demo dataset and runs through basic command line instructions and the corresponding results.

Who's there: MetaPhlAn2

- But don't! Instead, we've installed MetaPhlAn already for you by clicking here on the development site, http://bitbucket.org/biobakery/metaphlan2

Bitbucket Dashboard Teams Repositories Snippets Create Find a repository...

This repository's size is over 1 GB. Read more on how to reduce the size of your repository.

Overview

SSH ssh://hg@bitbucket.org/biobakery/metaph. Share

Last updated 2015-08-03
Language Python
Access level Admin (revoke)

1 Branch | 8 Tags | 2 Forks | 7 Watchers

Invite users to this repo
Send invitation

Recent activity

1 commit
Pushed to biobakery/metaphlan2
2ad8ff3 Bug fix. Bug resulting in execution...
Nicola Segata · 2015-08-03

3 commits
Pushed to biobakery/metaphlan2
183ae30 merge readme
dcd8820 add samout and heatmap to run_...
bdc26e4 add samout and heatmap to run_...
Duy Tin Truong · 2015-08-02

100% unclassified with Metaphlan2
Issue #8 commented on in biobakery/metaphlan2
Brittany Williams · 2015-07-28

1 commit
Pushed to biobakery/metaphlan2
2f454c1 README.md edited online with Bi...
Duy Tin Truong · 2015-07-28

1 commit
Pushed to biobakery/metaphlan2

Edit README

- MetaPhlAn 2.0: Metagenomic Phylogenetic Analysis
  - Description
  - Pre-requisites
  - Installation
  - Basic Usage
  - Full command-line options
  - Utility Scripts
    - Merging Tables
  - Heatmap Visualization
    - GraPhlAn Visualization
  - Customizing the database

MetaPhlAn 2.0: Metagenomic Phylogenetic Analysis

AUTHORS: Nicola Segata (nicola.segata@unitn.it)

Description

MetaPhlAn is a computational tool for profiling the composition of microbial communities (Bacteria, Archaea, Eukaryotes and Viruses) from metagenomic shotgun sequencing data with species level resolution. From version 2.0 MetaPhlAn is also able to identify specific strains (in the not-so-frequent cases in which the sample contains a previously sequenced strains) and to track strains across samples for all species.

MetaPhlAn 2.0 relies on ~1M unique clade-specific marker genes (the marker information file can be found at src/utils/markers_info.txt.bz2 or here) identified from ~17,000 reference genomes

Who's there: MetaPhlAn2

- The complete MetaPhlAn2 install is in `/class/stamps-software/biobakery/metaphlan2/`

Atlassian Bitbucket | Features | Pricing | owner/repository | English | Sign up | Log in

Source

default | MetaPhlAn2 /

db_v20			
utils			
.hgtags	205 B	4 hours ago	tagging version 2.0_beta3
README.md	24.6 KB	2 hours ago	README.md edited online with Bitbucket
metaphlan2.py	35.7 KB	6 hours ago	Making MetaPhlAn exiting graciously when the input format cannot be guessed because two files are

- MetaPhlAn 2.0: Metagenomic Phylogenetic Analysis
  - Description
  - Pre-requisites
  - Installation
  - Basic Usage
  - Full command-line options
  - Utility Scripts
    - Merging Tables
  - Heatmap Visualization
    - GraPhlAn Visualization

MetaPhlAn 2.0: Metagenomic Phylogenetic Analysis

From the command line...

- You can create your own virtual copy by running:

```
ln -s /home/ubuntu/metagenomics/metaphlan2/
```

- To see what you can do, run:

```
./metaphlan2/metaphlan2.py -h | less
```

  – Use the arrow keys to move up and down, `q` to quit back to the prompt

Who's there: MetaPhlAn2

1. ssh

```
usage: metaphlan2.py [-h] [-v] [--mpa_pkl] [--stat] [-t ANALYSIS TYPE]
                     [--tax_lev TAXONOMIC_LEVEL] [--nreads NUMBER_OF_READS]
                     [--pres_th PRESENCE_THRESHOLD]
                     [--bowtie2db METAPHLAN_BOWTIE2_DB]
                     [--bt2_ps BowTie2 presets] [--tmp_dir] [--clade]
                     [--min_ab] [--min_cu_len]
                     [--input_type {automatic,fastq,fasta,multifasta,multifastq,
bowtie2out,sam}]
                     [--ignore_viruses] [--ignore_eukaryotes]
                     [--ignore_bacteria] [--ignore_archaea] [--stat_q]
                     [--ignore_markers IGNORE_MARKERS] [--avoid_disqm]
                     [--bowtie2_exe BOWTIE2_EXE] [--bowtie2out FILE_NAME]
                     [--no_map] [-o output file] [--nproc N]
                     [--biom biom_output] [--mdelim mdelim]
                     [INPUT_FILE] [OUTPUT_FILE]

DESCRIPTION
 MetaPhlAn version 2.0.0 beta2 (12 July 2014): METAgenomic PHyLogenetic ANalysis
 for
 taxonomic classification of metagenomic reads.

AUTHORS: Nicola Segata (nicola.segata@unitn.it)

COMMON COMMANDS
:
```

Who's there: MetaPhlAn2

- To launch your first analysis, run:

```
./metaphlan2/metaphlan2.py \
    --mpa_pkl ./metaphlan2/db_v20/mpa_v20_m200.pkl \
    --bowtie2db ./metaphlan2/db_v20/mpa_v20_m200 \
    ./763577454-SRS014459-Stool.fasta \
    --input_type fasta \
    > ./763577454-SRS014459-Stool.txt
```

This will run for ~3-4 minutes

- What did you just do?
  - Two new output files:
  - `763577454-SRS014459-Stool.fasta.bowtie2out.txt`
    - Contains a mapping of reads to MetaPhlAn markers
  - `763577454-SRS014459-Stool.txt`
    - Contains taxonomic abundances as percentages

Who's there: MetaPhlAn2

```
less -S 763577454-SRS014459-Stool.fasta.bowtie2out.txt
```

1. [screen 2: bash] chuttenhower@class:~/tmp (ssh)

```
HWUSI-EAS1625_615HE:4:100:0:1248/1  gi|479140210|ref|NC_021010.1|:1043207-1044529
HWUSI-EAS1625_615HE:4:100:0:1301/1  gi|483877978|ref|NZ_KB890364.1|:31018-31902
HWUSI-EAS1625_615HE:4:100:1000:167/1    gi|242362078|ref|NZ_GG692716.1|:28261-29169
HWUSI-EAS1625_615HE:4:100:1001:1264/1   gi|270295698|ref|NZ_GG730107.1|:470181-472532
HWUSI-EAS1625_615HE:4:100:1001:1320/1   gi|224993849|ref|NZ_ACFY01000158.1|:c1296-10
HWUSI-EAS1625_615HE:4:100:1001:1604/1   gi|319644663|ref|NZ_GL635657.1|:c320982-320029
HWUSI-EAS1625_615HE:4:100:1001:1734/1   gi|484001485|ref|NZ_KB894131.1|:91019-91717
HWUSI-EAS1625_615HE:4:100:1001:259/1    gi|479210985|ref|NC_021043.1|:c1165057-1164158
HWUSI-EAS1625_615HE:4:100:1002:1501/1   gi|224485637|ref|NZ_EQ973491.1|:c620672-618312
HWUSI-EAS1625_615HE:4:100:1003:1644/1   gi|224485636|ref|NZ_EQ973490.1|:c204903-202990
HWUSI-EAS1625_615HE:4:100:1003:1702/1   gi|423335209|ref|NZ_JH976498.1|:329186-330046
HWUSI-EAS1625_615HE:4:100:1003:2030/1   gi|238922432|ref|NC_012781.1|:2910912-2912072
HWUSI-EAS1625_615HE:4:100:1004:353/1    gi|223955873|ref|NZ_DS499674.1|:c266282-265248
HWUSI-EAS1625_615HE:4:100:1004:742/1    gi|283767237|ref|NZ_GG730311.1|:c124395-124171
HWUSI-EAS1625_615HE:4:100:1005:1722/1   gi|410105720|ref|NZ_JH976502.1|:750498-751148
HWUSI-EAS1625_615HE:4:100:1005:505/1    gi|479170689|ref|NC_021020.1|:1540599-1542305
HWUSI-EAS1625_615HE:4:100:1006:848/1    gi|347530298|ref|NC_015977.1|:c3433030-3431387
HWUSI-EAS1625_615HE:4:100:1007:1428/1   gi|423332908|ref|NZ_JH976496.1|:1485161-1487113
HWUSI-EAS1625_615HE:4:100:1007:1465/1   gi|423332908|ref|NZ_JH976496.1|:906255-909584
HWUSI-EAS1625_615HE:4:100:1008:1187/1   gi|224485479|ref|NZ_EQ973214.1|:108053-108250
HWUSI-EAS1625_615HE:4:100:1008:1241/1   gi|270293478|ref|NZ_GG730105.1|:c830784-828727
HWUSI-EAS1625_615HE:4:100:1008:140/1    gi|224514921|ref|NZ_DS499545.1|:41991-42827
HWUSI-EAS1625_615HE:4:100:1009:154/1    gi|301307949|ref|NZ_GG774972.1|:644845-649113
HWUSI-EAS1625_615HE:4:100:1009:467/1    gi|303257489|ref|NZ_GL383997.1|:67163-67873
:
```

Who's there: MetaPhlAn2

```
less -S 763577454-SRS014459-Stool.txt
```

1. [screen 2: bash] chuttenhower@class:~/tmp (ssh)

```
k__Bacteria 100.0
k__Bacteria|p__Firmicutes   64.82041
k__Bacteria|p__Bacteroidetes    35.17959
k__Bacteria|p__Firmicutes|c__Clostridia 64.82041
k__Bacteria|p__Bacteroidetes|c__Bacteroidia 35.17959
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales    64.82041
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales    35.17959
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae 37.71449
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae  31.5000
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Eubacteriaceae  21.99035
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae 5.11557
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Porphyromonadaceae  3.6
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae|g__Subdolig
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacte
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Eubacteriaceae|g__Eubacteri
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__Roseburi
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Porphyromonadaceae|g__P
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae|g__Subdolig
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Eubacteriaceae|g__Eubacteri
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacte
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacte
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Eubacteriaceae|g__Eubacteri
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__Roseburi
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g__Bacte
763577454-SRS014459-Stool.txt
```

Who's there: MetaPhlAn2

- You can finish the job if you like:

```
./metaphlan2/metaphlan2.py \
    --mpa_pkl ./metaphlan2/db_v20/mpa_v20_m200.pkl \
    --bowtie2db ./metaphlan2/db_v20/mpa_v20_m200 \
    ./763577454-SRS014464-Anterior_nares.fasta \
    --input_type fasta \
    > ./763577454-SRS014464-Anterior_nares.txt
...
```

  – Note that you can use the up arrow key to make your life easier!

- Or you can copy the rest pre-calculated:

```
cp /home/ubuntu/metagenomics/results/metaphlan/*.txt .
```

Who's there: MetaPhlAn2

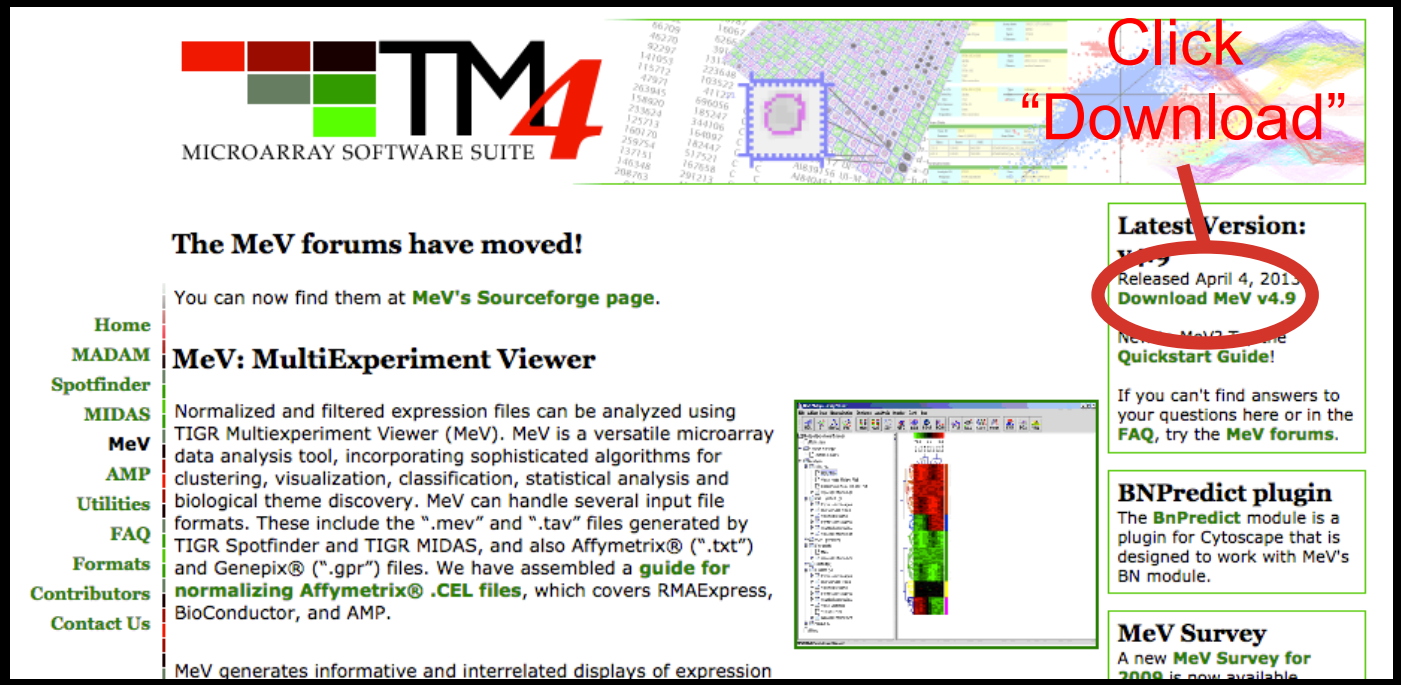
- Let's make a single table containing all six samples:

```
mkdir tmp
mv *.bowtie2out.txt tmp
./metaphlan2/utils/merge_metaphlan_tables.py *.txt > \
   763577454.tsv
```

- You can look at this file using `less`
  - Note 1: The arguments `less -x4 -S` will help
  - Note 2: You can set this "permanently" using `export LESS="-x4 -S"`

Who's there: MetaPhlAn

- But it's easier using MeV; go to http://www.tm4.org/mev.html

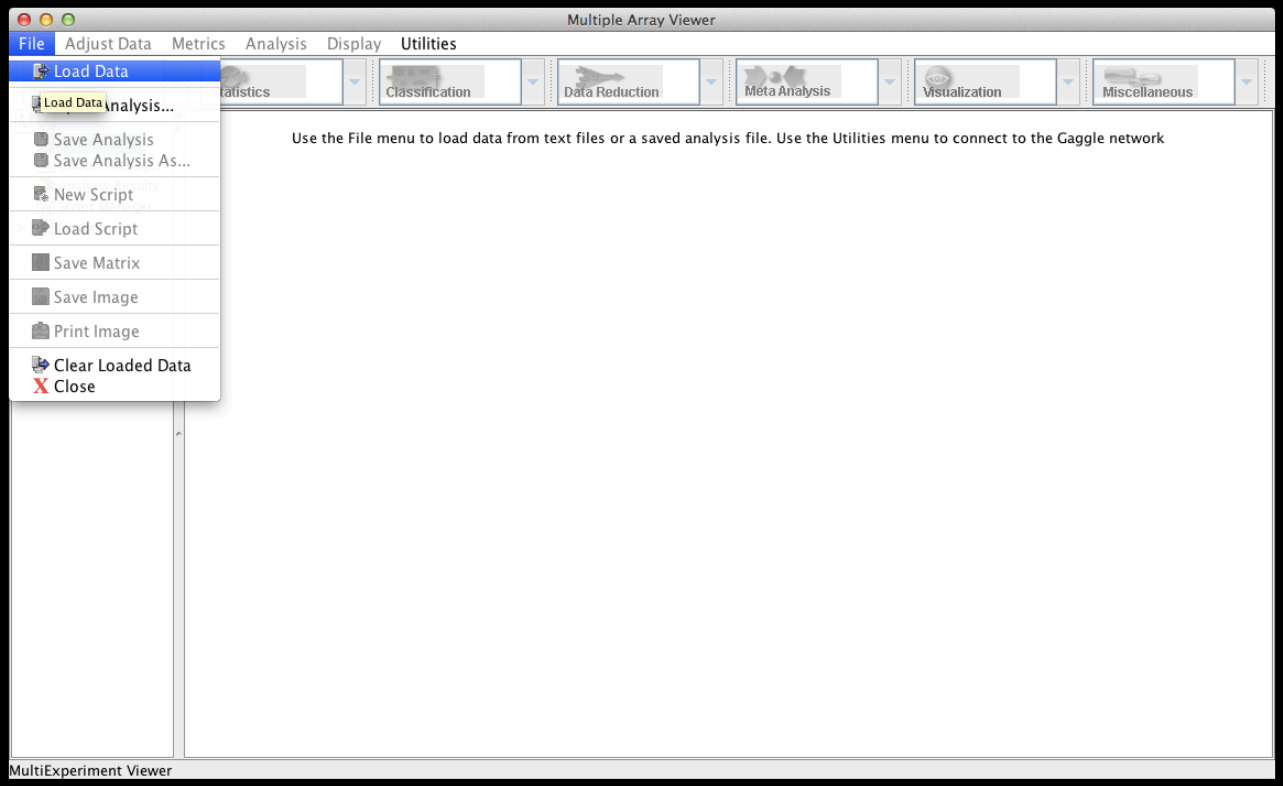


- Or use the appropriate local copy for your machine:

```
scp /home/ubuntu/metagenomics/ext/MeV_4_9_0_r2731_win.zip .
scp /home/ubuntu/metagenomics/ext/MeV_4_8_1_r2727_mac.tgz .
scp /home/ubuntu/metagenomics/ext/MeV_4_8_1_r2727_linux.tar.gz .
```

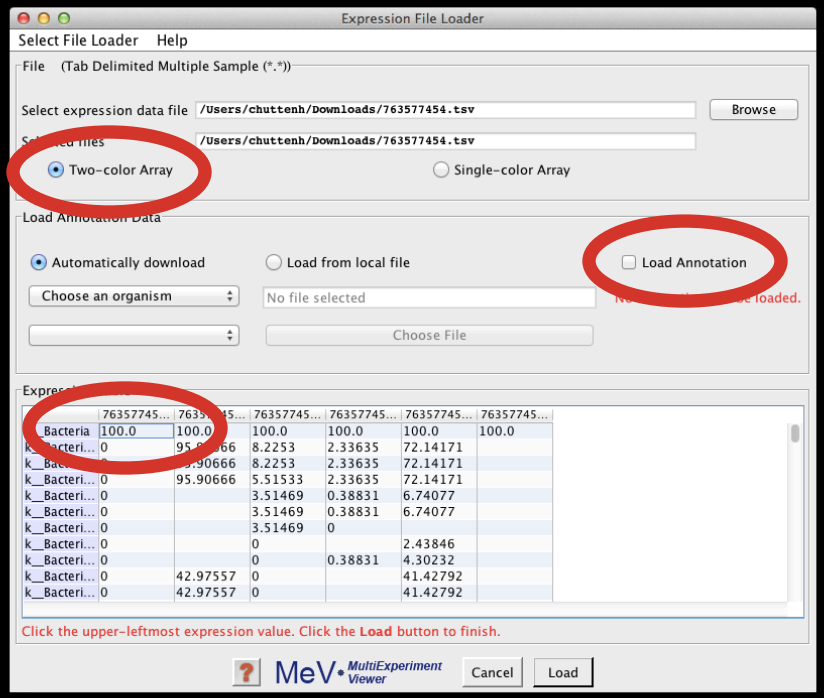
An interlude: MeV

- Don't forget to transfer your `763577454.tsv` file locally for viewing using `scp`
- Unzip, launch MeV, and select File/Load data



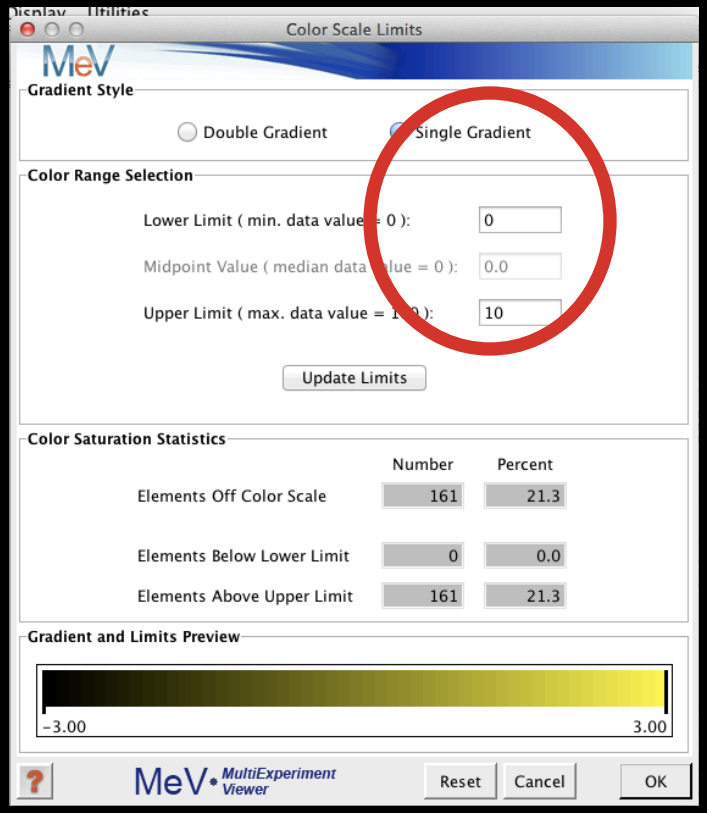
An interlude: MeV

- Click “Browse” to your TSV file, then
  - Tell MeV it’s a two-color array
  - Uncheck “Load annotation”
  - Click on the upper-leftmost ***data*** value



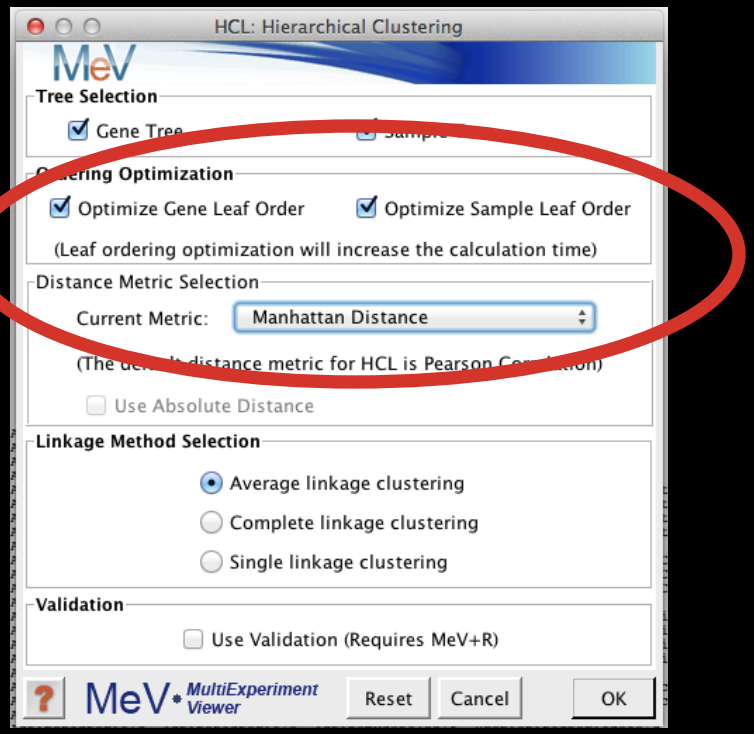
An interlude: MeV

- "Load" your data, then make it visible by:
  - Display/Set Color Scale Limits
  - Choose Single Gradient, min 0, max 10



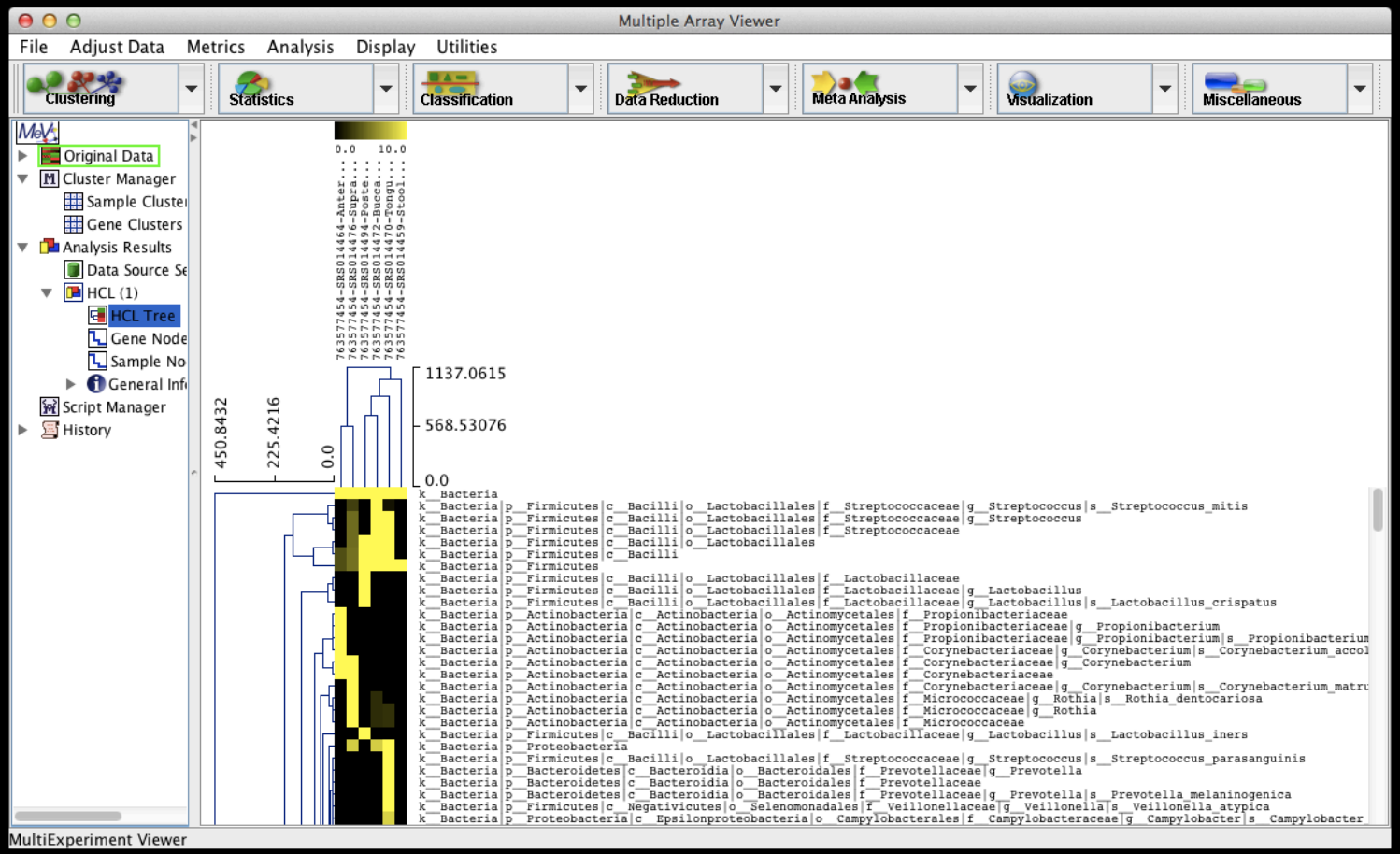
An interlude: MeV

- Finally, to play around a bit:
  - Display/Set Element Size/whatever you'd like
  - Clustering/Hierarchical Clustering
  - Optimize both gene and sample order
  - And select Manhattan Distance (imperfect!)



An interlude: MeV

- If you'd like, you can
  - Display/Sample-Column Labels/Abbr. Names



An interlude: MeV

- MeV is a tool; imperfect, but convenient
  - You should likely include just “leaf” nodes
    - Species, whose names start include “s__”
    - You can filter your file using:

```
cat 763577454.tsv | grep -E '(Stool)|(s__)' > \
      763577454_species.tsv
```

  - You can, but might not want to, z-score normalize
    - Adjust Data/Gene-Row Adjustments/Normalize Genes-Rows

- Many other tools built in – experiment!

Summary

- MetaPhlAn2
  - Evolution of MetaPhlAn1
    - Viruses, euks, subspecies, speed
    - And a LOT more reference data!
  - Raw metagenomic reads in
  - Tab-delimited species relative abundances out

Meta'omic functional profiling with ShortBRED

Galeb Abu-Ali
Eric Franzosa
Curtis Huttenhower

09-18-15

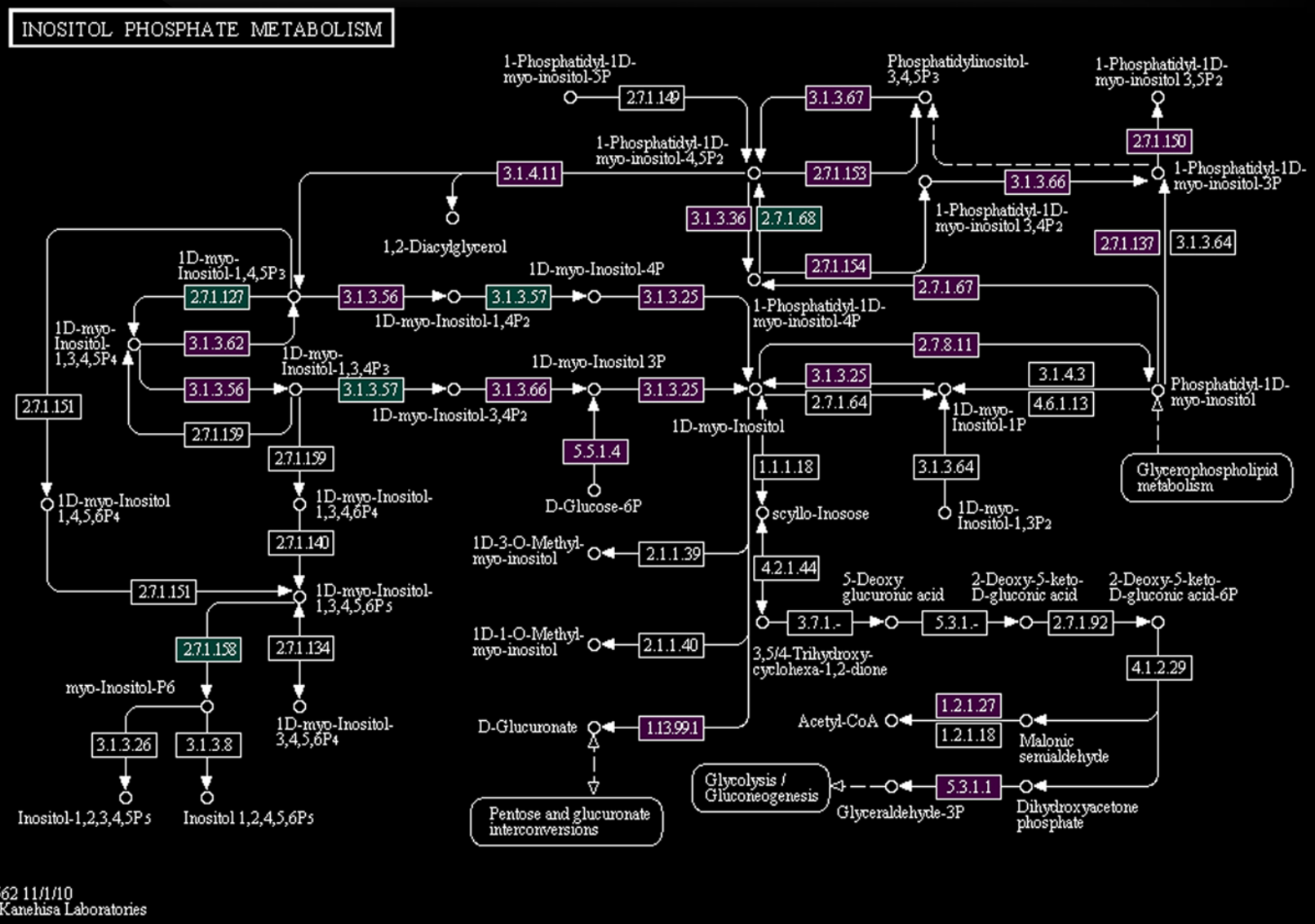
Harvard T.H. Chan School of Public Health
Department of Biostatistics

The two big questions…

Who is there?
(taxonomic profiling)

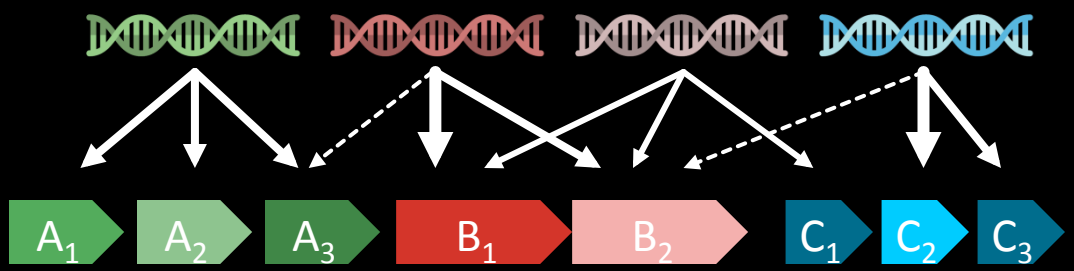
What are they doing?
(functional profiling)

(What we mean by "function")



HUMAnN

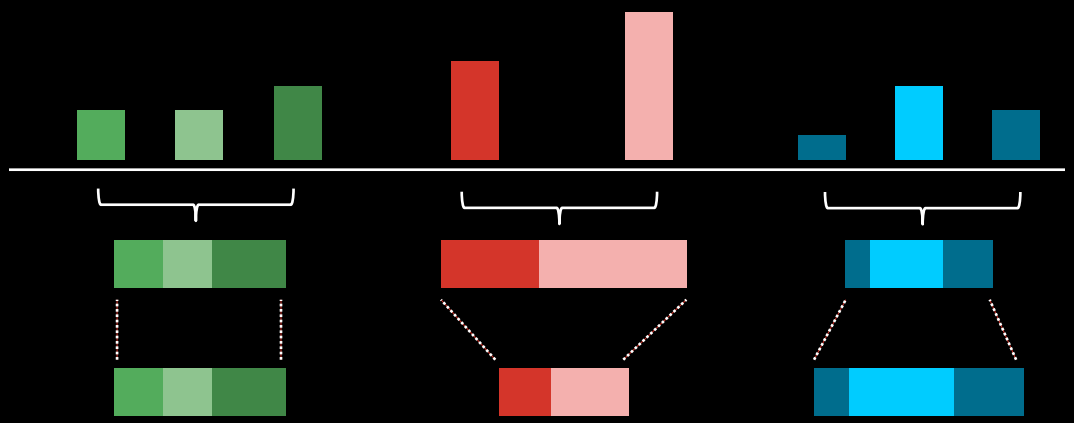
HMP Unified Metabolic Analysis Network



Short reads + protein families

Nucleotide pan-genome search

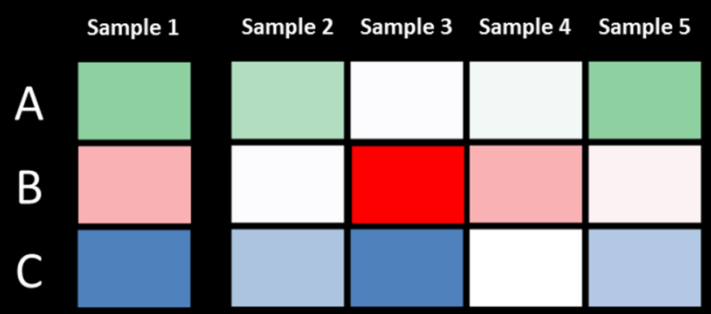
Translated BLAST search



Weight hits by %ID

Sum over seqs. within family

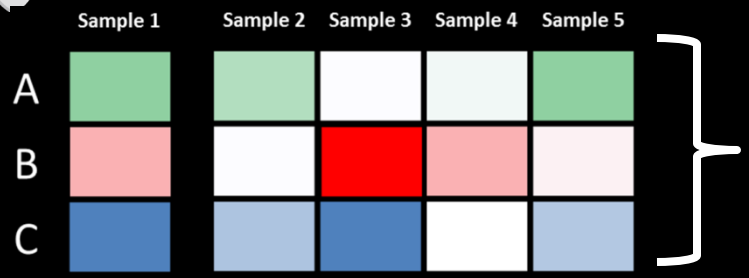
Adjust for sequence length



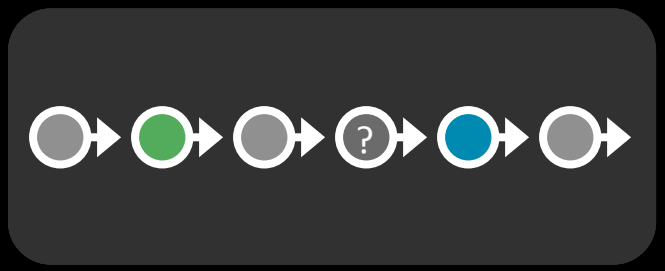
Repeat for each metagenomic or metatranscriptomic sample

HUMAnN

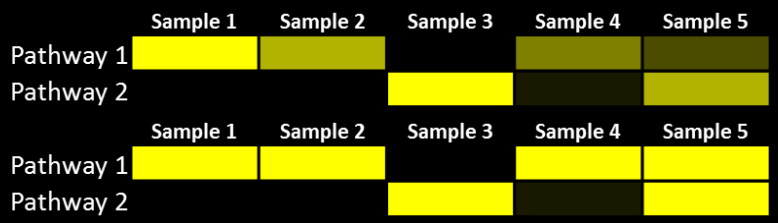
HMP Unified Metabolic Analysis Network



Many millions of hits are collapsed into a few million gene families (UniRefs) (*still a large number*)



- Map genes to MetaCyc pathways
- 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 UniRef abundance into MetaCyc pathway abundance (or presence/absence) yields a smaller, more tractable feature set

What's there: ShortBRED

Jim Kaminski

- **ShortBRED** is a tool for <u>quantifying protein families in metagenomes or metatranscriptomes</u>
  - Short Better REad Dataset

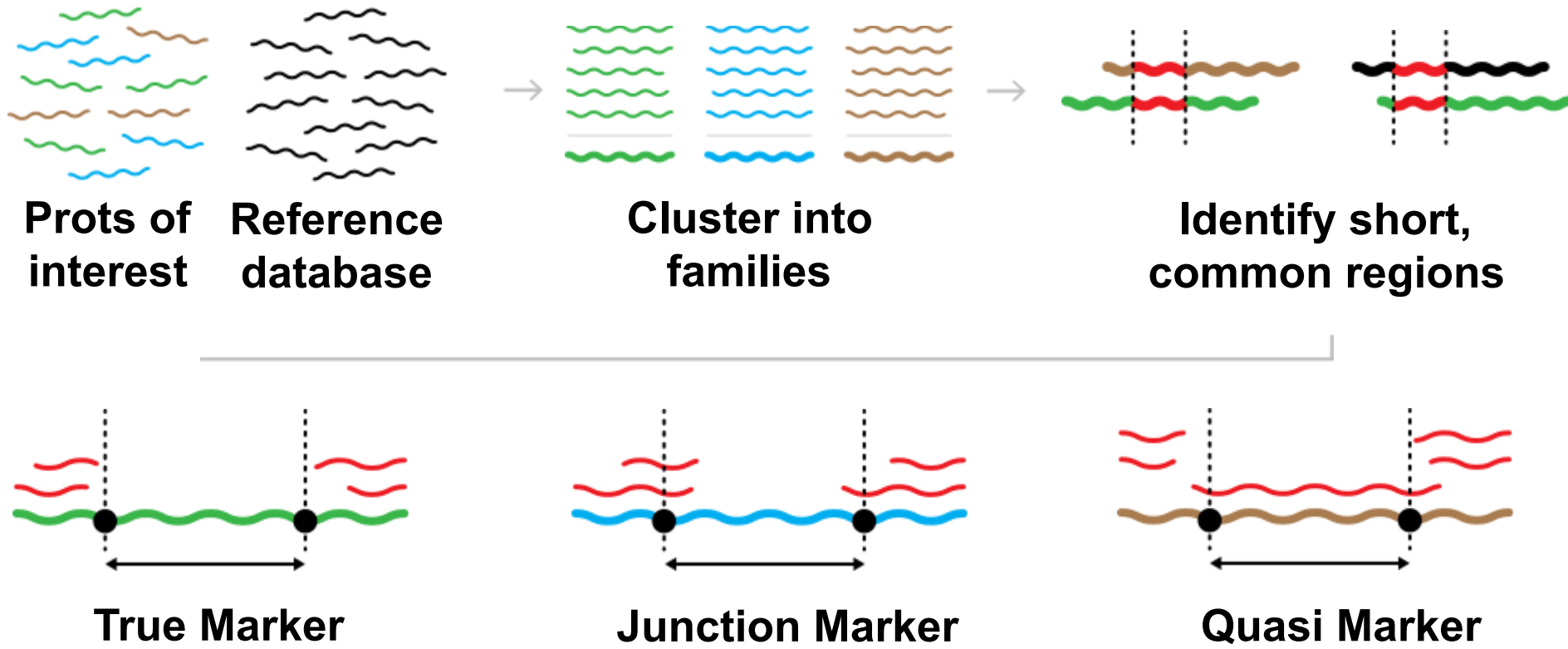
- Inputs:
  - FASTA file of proteins of interest
  - Large reference database of protein sequences (FASTA or blastdb)
  - Metagenomes (FASTA/FASTQ nucleotide files)
- Outputs:
  - Short, unique markers for protein families of interest (FASTA)
  - Relative abundances of protein families of interest in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

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

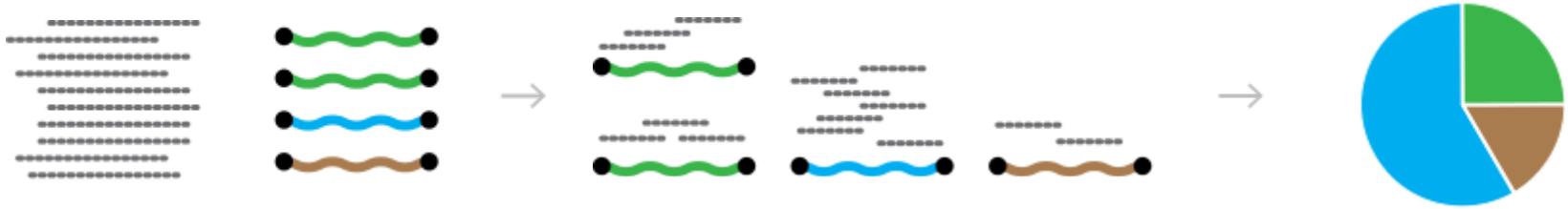
What's there: ShortBRED algorithm

- Cluster proteins of interest into families
  - Record consensus sequences
- Identify any 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



What's there: ShortBRED family quantification



Metagenome reads

ShortBRED markers

Translated search for high ID hits

Normalize relative abundances

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

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
- You can create your own virtual copy by running:

```
ln -s /home/ubuntu/metagenomics/shortbred/
```

- To see what you can do, run:

```
./shortbred/shortbred_identify.py -h | less -S
./shortbred/shortbred_quantify.py -h | less -S
```

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 /home/ubuntu/metagenomics/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

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

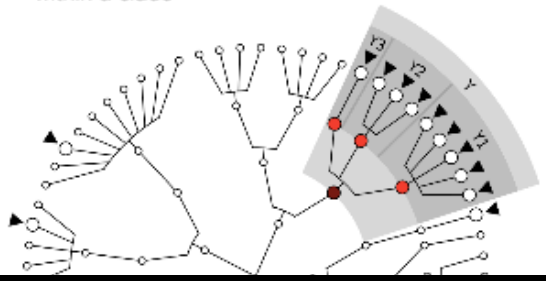
Streptococcus mitis

MetaRef Database v 1.0

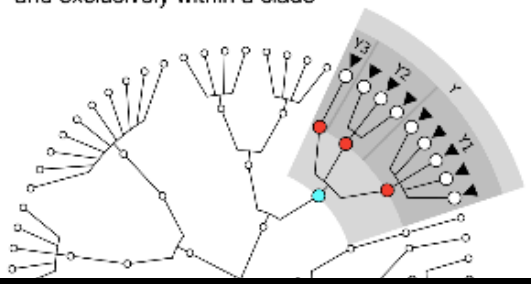
MetaRef is a resource to comprehensively catalog and characterize clade-specific microbial genes. We identify and provide all core genes associated with all microbial species and genera with available reference genomes (final or draft). A subset of these gene families are consistently present in one 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:

```
./shortbred/shortbred_identify.py \
    --goi /home/ubuntu/metagenomics/data/resisGenes.pfasta \
    --ref ./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:

`/home/ubuntu/metagenomics/results/shortbred/ardb_markers.faa`

  - It will produce lots of status output as it runs

ShortBRED markers

CFAR2015 — ubuntu@ip-10-113-166-56: ~/metagenomics/hutlabTest — ssh — 100×26

gabuali@hutlab...rray/slurm_logs3 | ubuntu@ip-10-...omics/hutlabTest | ubuntu@ip-10-1...mics/hutlabTest

```
>ZP_01723236_TM_#01
TEEFLGKYP
>ZP_01723236_TM_#02
IVVMWKRMLSLVGLYKIDGQSQSINRRFNLLHVIVGM
>ZP_01723236_TM_#03
FAFKDFIDDHLFKVEHVVYA
>ZP_01723236_TM_#04
KPKVDSLDKISYGLAF
>ZP_01723236_TM_#05
LVSVLKNWDTLSMDYFGFYAVGFISSFI
>ZP_01723236_TM_#06
ALISKVKLM
>AAA25717_TM_#01
MHLTITYWIDRLREAYPHAVAILLKGSYARGEASAWSDIDFDVLVSDEEVEEYRTWIEPV
GERLVHISVAVEWVTGWERDSADPSSWSYGLPTQETTQLLWAADENIRRRLDRPFKVHPA
AEPEVEDTVEALGKIRNAMVRGDDLAVYQAAQVVGKLIPTLLVPINPPTYARFAREAIDR
ILAFPNVPEGFAADWLTCMGLVDRRTHDPQPTRPNEWCAARSRFCRRMRTSSVRISRGCW
KQDWYLRISART
>CAD61201_TM_#01
MFQIRSFLVGISAFVMAVLGSAAYSAQPGGEYPTVDDIPVGEVRLYK
>CAD61201_TM_#02
LTRQLAEAAGNEVPAHSLKA
>CAD61201_TM_#03
AVRVLFGGCAVHEASRE
>CAD61201_TM_#04
ardb_markers.faa
```

True Markers
at the top

ShortBRED markers

CFAR2015 — ubuntu@ip-10-113-166-56: ~/metagenomics/hutlabTest — ssh — 100×26

gabuali@hutlab...rray/slurm_logs3 | ubuntu@ip-10-...omics/hutlabTest | ubuntu@ip-10-1...mics/hutlabTest

Junction/Quasi Markers
at the bottom

```
MNDIDREEPCAAAA
>P14509_TM_#02
PESMAAHVMGYKWARDKVGQSGCAVYRLHSKSGGSDLFLKHGKDAF
>P14509_TM_#03
GHISVPSVVSFVRTPNQAWLLTTAIHGKTAYQVLKSDFGARLVVVDALAAFMRRLHAIPV
SECS
>P14509_TM_#04
IEAGVVDVDDFDKEREGWTAEQVWEAMHRLLPLA
>P14509_TM_#05
LIVEGKVVGCIDVGRAGIADRYQDLAVLWNCLEEFEPSLQERLVAQYGIADPDRR
>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
>YP_970399_JM_#01__[YP_970399_w=0.306,ZP_03552050_w=0.163,YP_997055_w=0.163,YP_997055_w=0.102,CAJ939
47_w=0.061,YP_001348697_w=0.061,YP_316450_w=0.041,YP_002092118_w=0.061,Q2KX31_w=0.041]
GGMLLGLSRKAATDX
>ZP_01817983_JM_#01__[ZP_01817983_w=0.493,YP_001694417_w=0.362,YP_001694417_w=0.145]
TLTGPFIGGFIKEDFQPVAKEKAIPTKELFTSVK
(END)
```

Running ShortBRED-Quantify

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

```
./shortbred/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 take just a few seconds
  – It will again produce lots of status output as it runs

ShortBRED marker quantification

CFAR2015 — ubuntu@ip-10-113-166-56: ~/metagenomics/hutlabTest — ssh — 100×27

gabuali@hutlab...rray/slurm_logs3 | ubuntu@ip-10-...omics/hutlabTest | ubuntu@ip-10-1...mics/hutlabTest

Family	Count	Hits	TotMarkerLength
YP_001694417	2380.9523809523807	1	26
ZP_04679156	0.0	0	235
ZP_04657259	0.0	0	136
ZP_04635798	0.0	0	91
ZP_04635523	0.0	0	171
ZP_04633951	0.0	0	59
ZP_04616832	0.0	0	9
ZP_04613685	0.0	0	72
ZP_04606269	0.0	0	183
ZP_04577926	0.0	0	168
ZP_04543635	0.0	0	173
ZP_04543532	0.0	0	186
ZP_04433866	0.0	0	187
ZP_04431003	0.0	0	95
ZP_04405580	0.0	0	169
ZP_04405450	0.0	0	300
ZP_04309403	0.0	0	138
ZP_04284182	0.0	0	177
ZP_04244950	0.0	0	51
ZP_04210257	0.0	0	113
ZP_04197552	0.0	0	129
ZP_04175489	0.0	0	70
ZP_04174269	0.0	0	21
ZP_04151022	0.0	0	27
ZP_04107441	0.0	0	86

:

RPKMs and raw hit count

Other columns are family name and total AAs among all family makers

Sort table

```
(head -n 1; sort -k 2,2 -n -r) < \
   763577454-SRS014472-Buccal_mucosa-ARDB.txt | less
```

AR proteins in the human gut

- Example of some real data

  `/home/ubuntu/metagenomics/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 2012)
  - 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

Summary

- HUMAnN2 (up next!)
  - 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

Meta'omic functional profiling with HUMAnN2

Galeb Abu-Ali
Eric Franzosa
Curtis Huttenhower

09-18-15

Harvard School of Public Health
Department of Biostatistics

The two big questions…

Who is there?
(taxonomic profiling)

What are they doing?
(functional profiling)

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 they're doing: HUMAnN

- As a broad functional profiler, you *could* download HUMAnN at: http://huttenhower.sph.harvard.edu/humann

The Huttenhower Lab
Department of Biostatistics, Harvard School of Public Health

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

Home

HUMAnN: The HMP Unified Metabolic Analysis Network

Click here

You can obtain the HUMAnN software here:

humann-0.98.tar.gz

This is the latest version, which provided the analysis for [illegible] shotgun data from the Human Microbiome Project. If you find the software or data useful, please cite our manuscript:

Abubucker S, Segata N, Goll J, Schubert AM, Izard J, Cantarel BL, Rodriguez-Mueller B, Zucker J, Thiagarajan M, Henrissat B, White O, Kelley ST, Methé B, Schloss PD, Gevers D, Mitreva M, Huttenhower C. "Metabolic reconstruction for metagenomic data and its application to the human microbiome." PLoS Comput Biol. 2012 Jun;8(6):e1002358

Please contact us if you have any comments, suggestions, or bug reports for the software. Code is also available directly from our Mercurial source code repository at http://bitbucket.org/chuttenh/humann using the `hg clone` command.

HUMAnN is a pipeline for efficiently and accurately determining the presence/absence and abundance of microbial pathways in a community from metagenomic data. Sequencing a metagenome typically produces millions of short DNA/RNA reads. HUMAnN takes these reads as inputs and produces gene and pathway summaries as outputs:

- The abundance of each orthologous gene family in the community. Orthologous families are groups of genes that perform roughly the same biological roles. HUMAnN uses the KEGG Orthology (KO) by default, but any catalog of orthologs can be employed with minor changes (COG, NOG, etc.)
- The presence/absence of each pathway in the community. HUMAnN refers to pathway presence/absence as "coverage," and defines a pathway as a set of two or more genes. HUMAnN uses KEGG pathways and modules by default, but again can easily be modified to use GO terms or other gene sets.
- The abundance of each pathway in the community, i.e. how many "copies" of that pathway are present

What they're doing: HUMAnN2

- Or even *better*, the latest version is HUMAnN2 at: http://huttenhower.sph.harvard.edu/humann2

HUMAnN2: The HMP Unified Metabolic Analysis Network 2

HUMAnN2 is the next generation of HUMAnN. HUMAnN is a pipeline for efficiently and accurately profiling the presence/absence and abundance of microbial pathways in a community from metagenomic or metatranscriptomic sequencing data (typically millions of short DNA/RNA reads). This process, referred to as functional profiling, aims to describe the metabolic potential of a microbial community and its members. More generally, functional profiling answers the question "What are the microbes in my community-of-interest doing (or capable of doing)?"

If you use the HUMAnN2 software, please cite our manuscript: TBD

For additional information, please see the HUMAnN2 User Manual.

Contents

Click here

What they're doing: HUMAnN2

- ...but instead we've already installed it!
- Normally you'd follow the online tutorial to expand:

```
tar -xzf humann2_v0.2.3.tar.gz
```

- Install:

```
cd humann2_v0.2.3
python setup.py minpath
python setup.py install
```

- And download DIAMOND from here:
  - http://ab.inf.uni-tuebingen.de/software/diamond/

We're going to use it preinstalled instead

What they're doing: HUMAnN2

- If we weren't all running this, you'd need to:
  - Get our precomputed DNA/AA databases
    - ChocoPhlAn ~50M genes from NCBI
    - UniRef ~100M proteins from UniProt

```
humann2_databases --download chocophlan full \
   /class/stamps-software/biobakery/humann2/
humann2_databases --download uniref diamond \
   /class/stamps-software/biobakery/humann2/
```

- This would take too long for everyone to use, so we'll stick with the demo database instead...

What they're doing: HUMAnN2

- Take a look at the demo input metagenome:

```
less -S /home/ubuntu/metagenomics/data/humann2/examples/demo.fastq
```

- From your home directory, run HUMAnN2:

```
humann2 \
    --input /home/ubuntu/metagenomics/data/humann2/examples/
demo.fastq \
    --output humann2_demo
```

- What did you just do?

```
less -S humann2_demo/demo_genefamilies.tsv
```

  – UniRef gene family IDs
  – With human-readable glosses when available
  – Broken down per organism

What they're doing: HUMAnN2

CFAR2015 — ubuntu@ip-10-113-166-56: ~/metagenomics/hutlabTest — ssh — 100×32

gabuali@hutlab...rray/slurm_logs3 | ubuntu@ip-10-...omics/hutlabTest | ubuntu@ip-10-113-166-56: ~

```
# Gene Family   demo_Abundance
UniRef50_A6L108 8.7719298246
UniRef50_A6L108|g__Bacteroides.s__Bacteroides_stercoris 8.7719298246
UniRef50_E1WMC2 7.5757575758
UniRef50_E1WMC2|g__Bacteroides.s__Bacteroides_fragilis  7.5757575758
UniRef50_R5FJB9: Conjugative transposon TraN protein    7.3070013559
UniRef50_R5FJB9: Conjugative transposon TraN protein|g__Bacteroides.s__Bacteroides_fragilis     3.05
UniRef50_R5FJB9: Conjugative transposon TraN protein|g__Bacteroides.s__Bacteroides_thetaiotaomicron
UniRef50_R5FJB9: Conjugative transposon TraN protein|g__Bacteroides.s__Bacteroides_stercoris    2.07
UniRef50_B6YQ01: 50S ribosomal protein L11      6.7567567568
UniRef50_B6YQ01: 50S ribosomal protein L11|g__Bacteroides.s__Bacteroides_fragilis       2.2522522523
UniRef50_B6YQ01: 50S ribosomal protein L11|g__Bacteroides.s__Bacteroides_stercoris      2.2522522523
UniRef50_B6YQ01: 50S ribosomal protein L11|g__Bacteroides.s__Bacteroides_thetaiotaomicron       2.25
UniRef50_Q64R13 6.5359477124
UniRef50_Q64R13|g__Bacteroides.s__Bacteroides_fragilis  6.5359477124
UniRef50_F5XD83: Conjugative transposon protein TraK    6.4102564103
UniRef50_F5XD83: Conjugative transposon protein TraK|g__Bacteroides.s__Bacteroides_thetaiotaomicron
UniRef50_F5XD83: Conjugative transposon protein TraK|g__Bacteroides.s__Bacteroides_fragilis     1.60
UniRef50_F5XD83: Conjugative transposon protein TraK|g__Bacteroides.s__Bacteroides_stercoris    1.60
UniRef50_B6YQ88: 30S ribosomal protein S7       6.2893081761
UniRef50_B6YQ88: 30S ribosomal protein S7|g__Bacteroides.s__Bacteroides_stercoris       4.1928721174
UniRef50_B6YQ88: 30S ribosomal protein S7|g__Bacteroides.s__Bacteroides_fragilis        2.0964360587
UniRef50_D1KAI8 6.2893081761
UniRef50_D1KAI8|g__Bacteroides.s__Bacteroides_stercoris 6.2893081761
UniRef50_Q2S3Q3: 50S ribosomal protein L24      6.2599188856
UniRef50_Q2S3Q3: 50S ribosomal protein L24|g__Bacteroides.s__Bacteroides_thetaiotaomicron       3.14
UniRef50_Q2S3Q3: 50S ribosomal protein L24|g__Bacteroides.s__Bacteroides_stercoris      3.1152647975
UniRef50_A0A016LIR2     6.1728395062
UniRef50_A0A016LIR2|g__Bacteroides.s__Bacteroides_stercoris     6.1728395062
UniRef50_Q5LEY5 6.1728395062
UniRef50_Q5LEY5|g__Bacteroides.s__Bacteroides_fragilis  6.1728395062
humann2_demo/demo_genefamilies.tsv
```

What they're doing: HUMAnN2

- This has created three main files:
  - One listing gene family abundances
  - Two listing pathway (default MetaCyc) abundances and coverages
    - Coverage % of "essential" pathway genes present
    - Abundance "Average" abundance of essential pathway genes

- Each is tab-delimited text with two columns

`humann2_demo/demo_genefamilies.tsv`
- Relative abundance (RPKM) of gene families (UniRef)

`humann2_demo/demo_pathabundance.tsv`
- Relative abundance (RPKM) of pathways (MetaCyc)

`humann2_demo/demo_pathacoverage.tsv`
- Coverage (%) of pathways (MetaCyc)

- I almost always just use abundances (gene or pathway)

What they're doing: HUMAnN2

- Pathways look very much like gene families:

```
less -S humann2_demo/demo_pathabundance.tsv
```

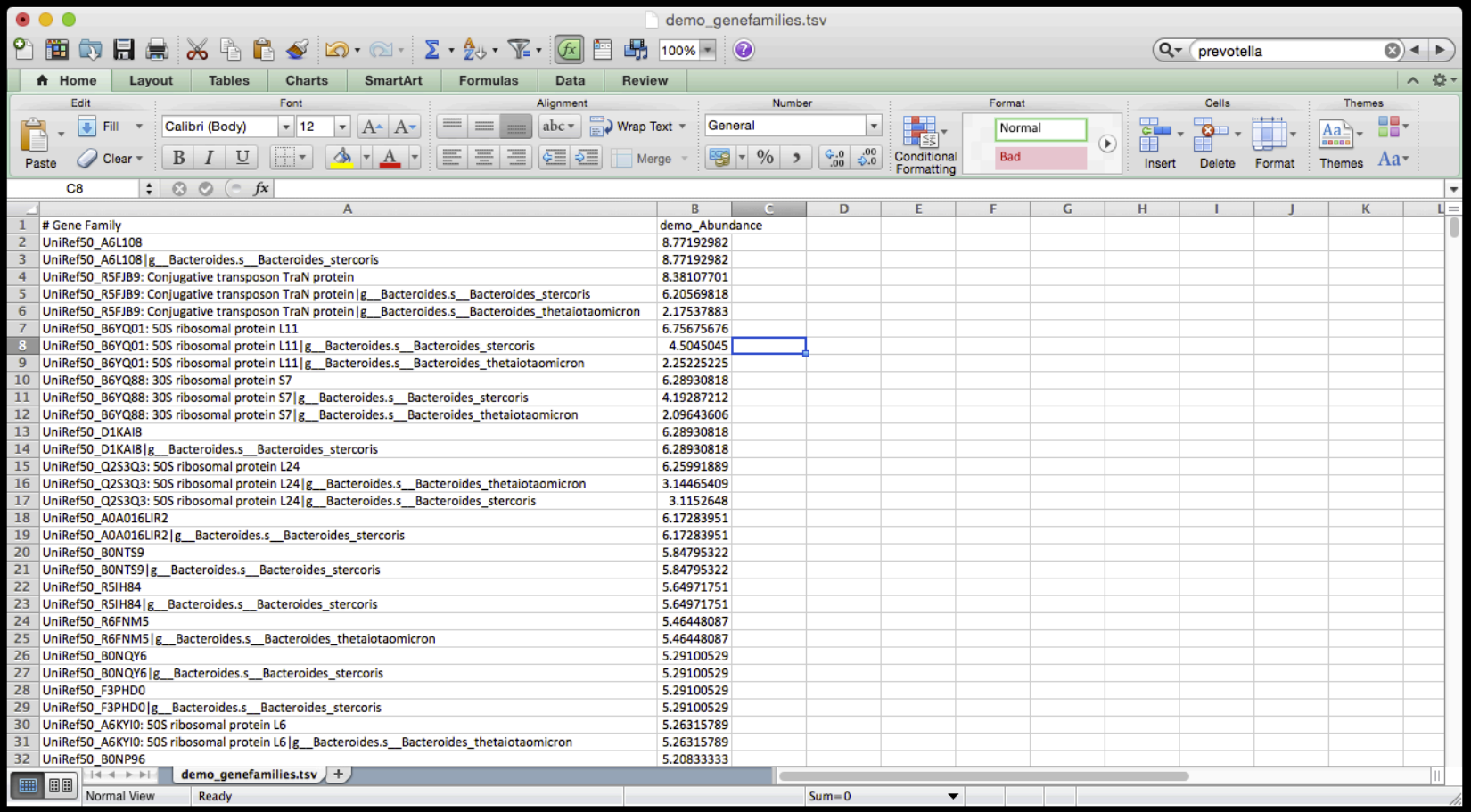
CFAR2015 — ubuntu@ip-10-113-166-56: ~/metagenomics/hutlabTest — ssh — 100×24

gabuali@hutlab...rray/slurm_logs3 | ubuntu@ip-10-...omics/hutlabTest | ubuntu@ip-10-113-166-56: ~

```
# Pathway        demo_Abundance
SUCROSEUTIL2-PWY: sucrose degradation VII (sucrose 3-dehydrogenase)     5.8949329489
SUCROSEUTIL2-PWY: sucrose degradation VII (sucrose 3-dehydrogenase)|g__Bacteroides.s__Bacteroides_th
SUCROSEUTIL2-PWY: sucrose degradation VII (sucrose 3-dehydrogenase)|g__Bacteroides.s__Bacteroides_st
PWY-6627: salinosporamide A biosynthesis        2.8307602366
PWY-6627: salinosporamide A biosynthesis|g__Bacteroides.s__Bacteroides_thetaiotaomicron 0.9701665013
PWY-5209: methyl-coenzyme M oxidation to CO2    2.7862228006
PWY-7555: &alpha;-cyclopiazonate biosynthesis   2.6676145598
PWY-7555: &alpha;-cyclopiazonate biosynthesis|g__Bacteroides.s__Bacteroides_thetaiotaomicron    0.86
PWY-7440: dTDP-&beta;-L-4-epi-vancosamine biosynthesis  2.5755106274
PWY-3841: folate transformations II     2.3673882669
PWY-7301: dTDP-&beta;-L-noviose biosynthesis    2.3051941136
PWY-7043: 11-cis-3-hydroxyretinal biosynthesis  2.3017477039
PWY-7043: 11-cis-3-hydroxyretinal biosynthesis|g__Bacteroides.s__Bacteroides_stercoris  2.0827022653
HSERMETANA-PWY: L-methionine biosynthesis III   2.2932930562
HSERMETANA-PWY: L-methionine biosynthesis III|g__Bacteroides.s__Bacteroides_thetaiotaomicron    0.99
PWY-7104: dTDP-L-megosamine biosynthesis        2.2089022269
PWY-5100: pyruvate fermentation to acetate and lactate II       2.1231927723
PWY-5100: pyruvate fermentation to acetate and lactate II|g__Bacteroides.s__Bacteroides_stercoris
PWY-7432: L-phenylalanine biosynthesis III (cytosolic, plants)  2.0812499269
PWY-7432: L-phenylalanine biosynthesis III (cytosolic, plants)|g__Bacteroides.s__Bacteroides_thetaio
PWY-6973: dTDP-D-olivose, dTDP-D-oliose and dTDP-D-mycarose biosynthesis        1.9963472394
PWY-6973: dTDP-D-olivose, dTDP-D-oliose and dTDP-D-mycarose biosynthesis|g__Bacteroides.s__Bacteroid
humann2_demo/demo_pathabundance.tsv
```

What they're doing: HUMAnN2

- You can always open these in Excel too
  - **Note:** this is sparse since we're using small subsets of the reference data (ChocoPhlAn and UniRef) and input metagenome



# Gene Family	demo_Abundance
UniRef50_A6L108	8.77192982
UniRef50_A6L108|g__Bacteroides.s__Bacteroides_stercoris	8.77192982
UniRef50_R5FJB9: Conjugative transposon TraN protein	8.38107701
UniRef50_R5FJB9: Conjugative transposon TraN protein|g__Bacteroides.s__Bacteroides_stercoris	6.20569818
UniRef50_R5FJB9: Conjugative transposon TraN protein|g__Bacteroides.s__Bacteroides_thetaiotaomicron	2.17537883
UniRef50_B6YQ01: 50S ribosomal protein L11	6.75675676
UniRef50_B6YQ01: 50S ribosomal protein L11|g__Bacteroides.s__Bacteroides_stercoris	4.5045045
UniRef50_B6YQ01: 50S ribosomal protein L11|g__Bacteroides.s__Bacteroides_thetaiotaomicron	2.25225225
UniRef50_B6YQ88: 30S ribosomal protein S7	6.28930818
UniRef50_B6YQ88: 30S ribosomal protein S7|g__Bacteroides.s__Bacteroides_stercoris	4.19287212
UniRef50_B6YQ88: 30S ribosomal protein S7|g__Bacteroides.s__Bacteroides_thetaiotaomicron	2.09643606
UniRef50_D1KAI8	6.28930818
UniRef50_D1KAI8|g__Bacteroides.s__Bacteroides_stercoris	6.28930818
UniRef50_Q2S3Q3: 50S ribosomal protein L24	6.25991889
UniRef50_Q2S3Q3: 50S ribosomal protein L24|g__Bacteroides.s__Bacteroides_thetaiotaomicron	3.14465409
UniRef50_Q2S3Q3: 50S ribosomal protein L24|g__Bacteroides.s__Bacteroides_stercoris	3.1152648
UniRef50_A0A016LIR2	6.17283951
UniRef50_A0A016LIR2|g__Bacteroides.s__Bacteroides_stercoris	6.17283951
UniRef50_B0NTS9	5.84795322
UniRef50_B0NTS9|g__Bacteroides.s__Bacteroides_stercoris	5.84795322
UniRef50_R5IH84	5.64971751
UniRef50_R5IH84|g__Bacteroides.s__Bacteroides_stercoris	5.64971751
UniRef50_R6FNM5	5.46448087
UniRef50_R6FNM5|g__Bacteroides.s__Bacteroides_thetaiotaomicron	5.46448087
UniRef50_B0NQY6	5.29100529
UniRef50_B0NQY6|g__Bacteroides.s__Bacteroides_stercoris	5.29100529
UniRef50_F3PHD0	5.29100529
UniRef50_F3PHD0|g__Bacteroides.s__Bacteroides_stercoris	5.29100529
UniRef50_A6KYI0: 50S ribosomal protein L6	5.26315789
UniRef50_A6KYI0: 50S ribosomal protein L6|g__Bacteroides.s__Bacteroides_thetaiotaomicron	5.26315789
UniRef50_B0NP96	5.20833333

What they're doing: HUMAnN2

- If you run more than one sample, you can combine them:

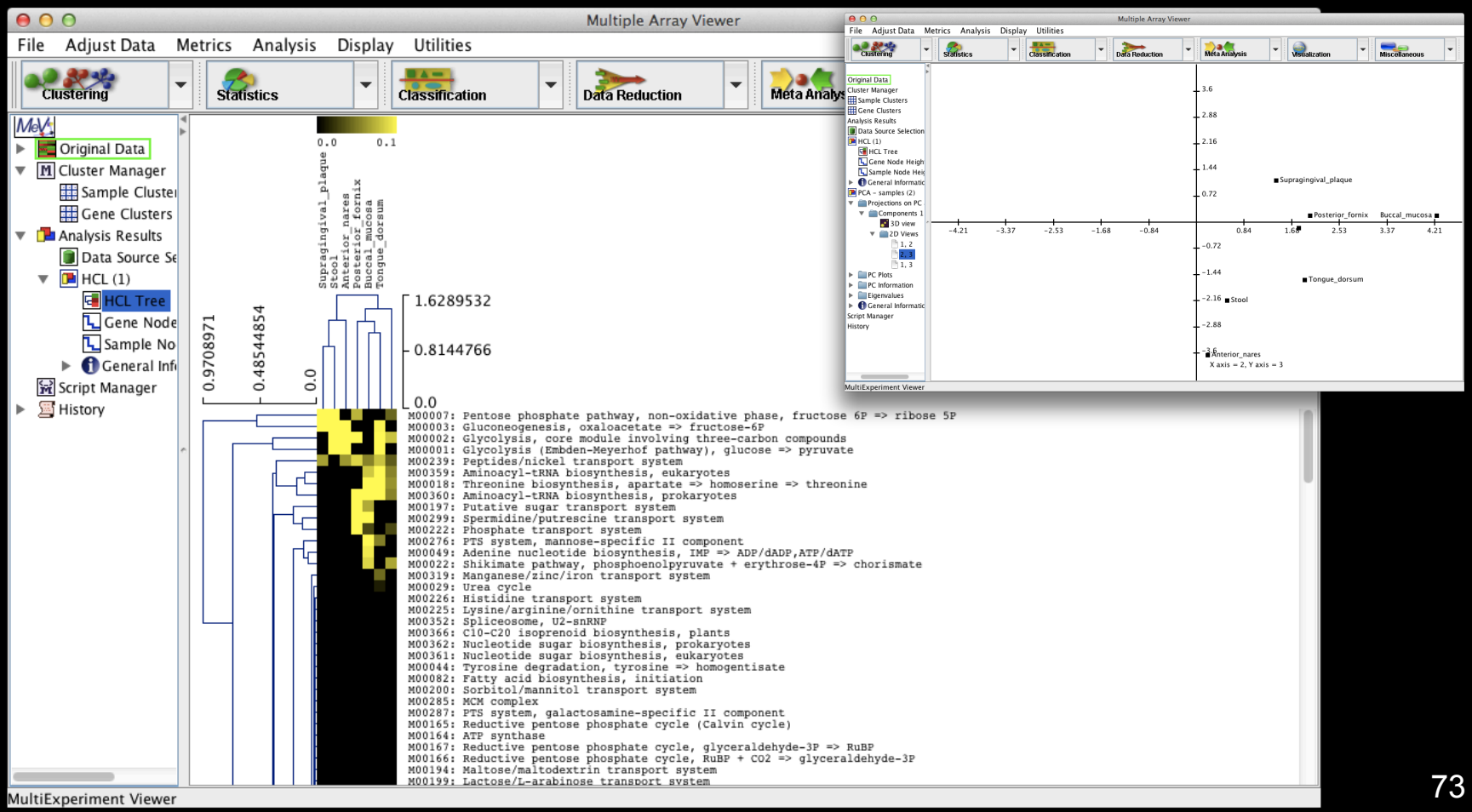
```
less -S \
     /home/ubuntu/metagenomics/data/humann2/genes/763577454-SRS014459-Stool_genefamilies.tsv
/home/ubuntu/metagenomics/data/humann2/humann2/tools/join_tables.py \
     -i /home/ubuntu/metagenomics/data/humann2/genes/ \
     -o 763577454_genefamilies.tsv
less -S 763577454_genefamilies.tsv
```

- And you can open the resulting table in Excel/etc.

# Gene Family	763577454-S	763577454-S	763577454-S	763577454-S	763577454-S	763577454-SRS014494-Posterior_fornix_Abundance
UniRef50_A9FGD2: 50S ribosomal protein L36	17.0940171	0	0	0	0	0
UniRef50_A9FGD2: 50S ribosomal protein L36|g__Bacteroides.s__Bacteroides_cellulosilyticus	17.0940171	0	0	0	0	0
UniRef50_R6AES1	14.8148148	0	0	0	0	0
UniRef50_R6AES1|g__Bacteroides.s__Bacteroides_stercoris	14.8148148	0	0	0	0	0
UniRef50_UPI00047E7990: glycosyl transferase family 1	0	8.96495518	0	0.81499593	3.2599837	1.05317188
UniRef50_UPI00047E7990: glycosyl transferase family 1|unclassified	0	8.96495518	0	0.81499593	3.2599837	1.05317188
UniRef50_UPI00047498D2: hypothetical protein, partial	3.003003	3.003003	3.003003	3.003003	0	0
UniRef50_UPI00047498D2: hypothetical protein, partial|unclassified	3.003003	3.003003	3.003003	3.003003	0	0
UniRef50_A6L108	11.6959064	0	0	0	0	0
UniRef50_A6L108|g__Bacteroides.s__Bacteroides_stercoris	11.6959064	0	0	0	0	0
UniRef50_R6Q0V8: ABC-type metal ion transport system periplasmic component/surface antigen	3.23624595	0	2.1574973	2.1574973	3.23624595	0
UniRef50_R6Q0V8: ABC-type metal ion transport system periplasmic component/surface antigen|unclassified	3.23624595	0	2.1574973	2.1574973	3.23624595	0
UniRef50_E6UAV0: Preprotein translocase, YajC subunit	2.94985251	0	0.98420138	5.89970501	0.65314007	0
UniRef50_E6UAV0: Preprotein translocase, YajC subunit|unclassified	2.94985251	0	0.98420138	5.89970501	0.65314007	0
UniRef50_U2Q6I3	0	0	0	5.20833333	0	5.20833333
UniRef50_U2Q6I3|unclassified	0	0	0	5.20833333	0	5.20833333
UniRef50_A6KXA8: Transposase	10.0704935	0	0	0	0	0
UniRef50_A6KXA8: Transposase|g__Bacteroides.s__Bacteroides_stercoris	10.0704935	0	0	0	0	0
UniRef50_UPI000374C24F: hypothetical protein	1.68740089	0	3.23238577	0	4.67294834	0
UniRef50_UPI000374C24F: hypothetical protein|unclassified	1.68740089	0	3.23238577	0	4.67294834	0
UniRef50_P37247: Transposase for insertion sequence element IS4351	9.17431193	0	0	0	0	0
UniRef50_P37247: Transposase for insertion sequence element IS4351|g__Bacteroides.s__Bacteroides_cellulosilyticus	9.17431193	0	0	0	0	0
UniRef50_B0NNQ2: Transposase	9.00031022	0	0	0	0	0
UniRef50_B0NNQ2: Transposase|g__Bacteroides.s__Bacteroides_stercoris	9.00031022	0	0	0	0	0
UniRef50_R0KTN8	0	0	1.51860289	1.51860289	3.8238261	1.79224317
UniRef50_R0KTN8|unclassified	0	0	1.51860289	1.51860289	3.8238261	1.79224317

What they're doing: HUMAnN2

- And there's nothing stopping us from using MeV
  - Or R, or QIIME, or LEfSe, or anything that'll read tab-delimited text



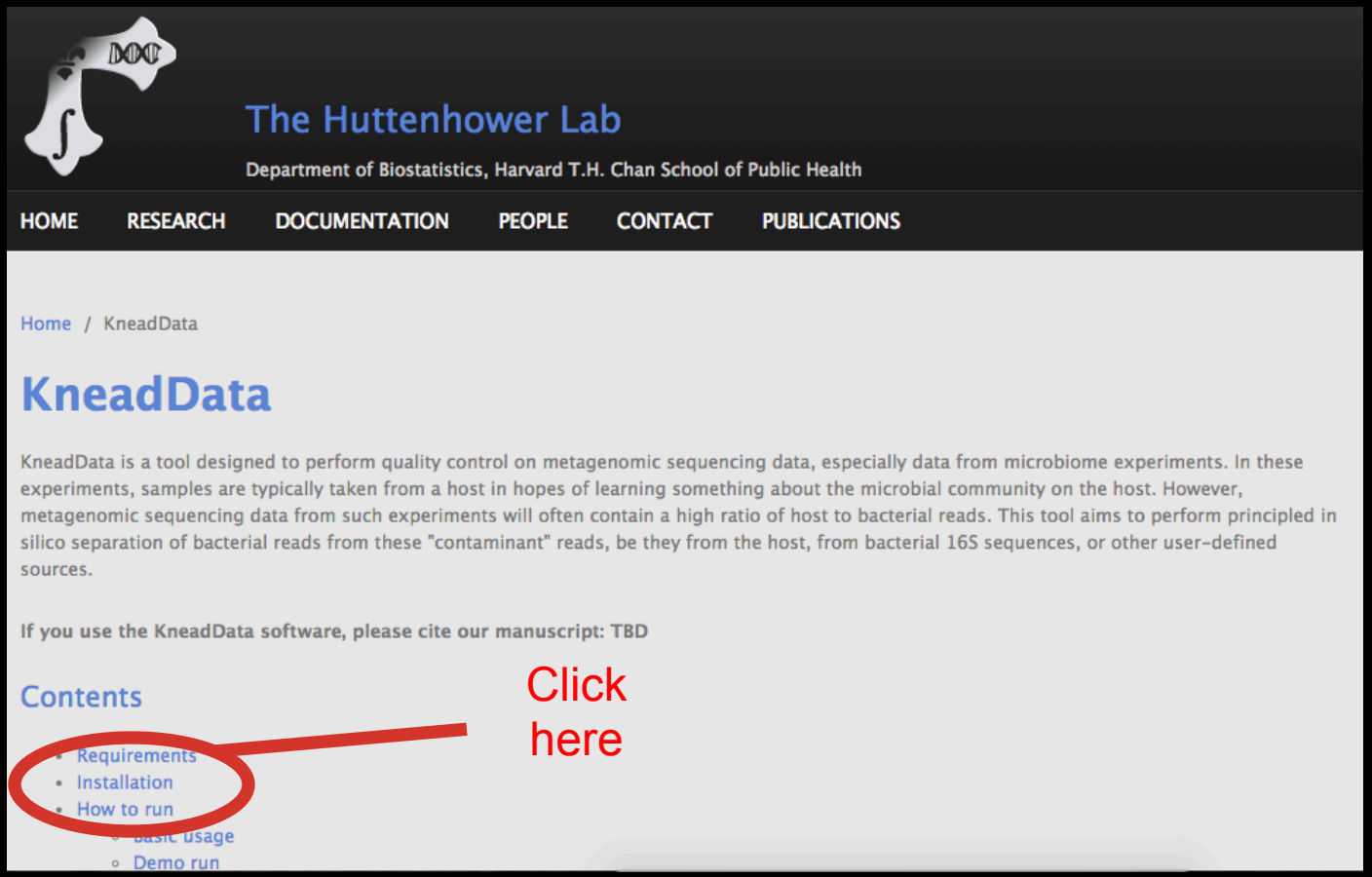
Quality control: KneadData

- Did you notice that we didn't QC our data at all?
  - MetaPhlAn2 is very robust to junk sequence
  - HUMAnN2 is pretty robust, but not quite as much

- Demo data includes standard metagenomic QC:
  - Quality trim by removing bad bases (typically Q ~15)
  - Length filter to remove short sequences (typically <75%)

Metagenome and metatranscriptome quality control: KneadData

- You can trim and filter reads, remove host contamination, and deplete ribosomal sequences using: http://huttenhower.sph.harvard.edu/kneaddata



Metagenome and metatranscriptome quality control: KneadData

- KneadData performs quality trimming using Trimmomatic:

```
kneaddata -1 seq1.fastq -a SLIDINGWINDOW:4:20 -o seqs
```

- And read length filtering (including paired ends):

```
kneaddata -1 seq1.fastq -2 seq2.fastq \
    -a "SLIDINGWINDOW:4:20 MINLEN:60" -o seqs
```

- And will remove host (e.g. human) sequences from a reference database:

```
kneaddata -1 seq1.fastq -2 seq2.fastq \
    -a "SLIDINGWINDOW:4:20 MINLEN:60" \
    -db Homo_sapiens_db -o seqs
```

- And will remove ribosomal sequences (for metatranscriptomes):

```
kneaddata -1 seq1.fastq -2 seq2.fastq \
    -a "SLIDINGWINDOW:4:20 MINLEN:60" \
    -db Homo_sapiens_db -db bact_rrna_db -o seqs
```

Multivariate associating testing with random effects using MaAsLin

Galeb Abu-Ali
Eric Franzosa
Curtis Huttenhower

09-18-15

Harvard School of Public Health
Department of Biostatistics

The ~~two~~ three big questions…

Who is there?

What are they doing?

What does it all mean?

Sample #	1	2	3	4	5	6
Profession	Student	Postdoc	Postdoc	Professor	Student	Student
Gender	Male	Female	Female	Male	Male	Female
Site	Oral	Gut	Oral	Gut	Oral	Gut
Clade1	0.40	0.87	0.43	0.68	0.47	0.32
Clade1|Bug1	0.40	0.56	0.07	0.31	0.42	0.27
Clade1|Bug2	0.00	0.30	0.36	0.37	0.04	0.05
Clade2	0.60	0.13	0.57	0.32	0.53	0.68
Clade2|Bug3	0.11	0.00	0.10	0.32	0.15	0.23
Clade2|Bug4	0.49	0.13	0.47	0.00	0.39	0.45

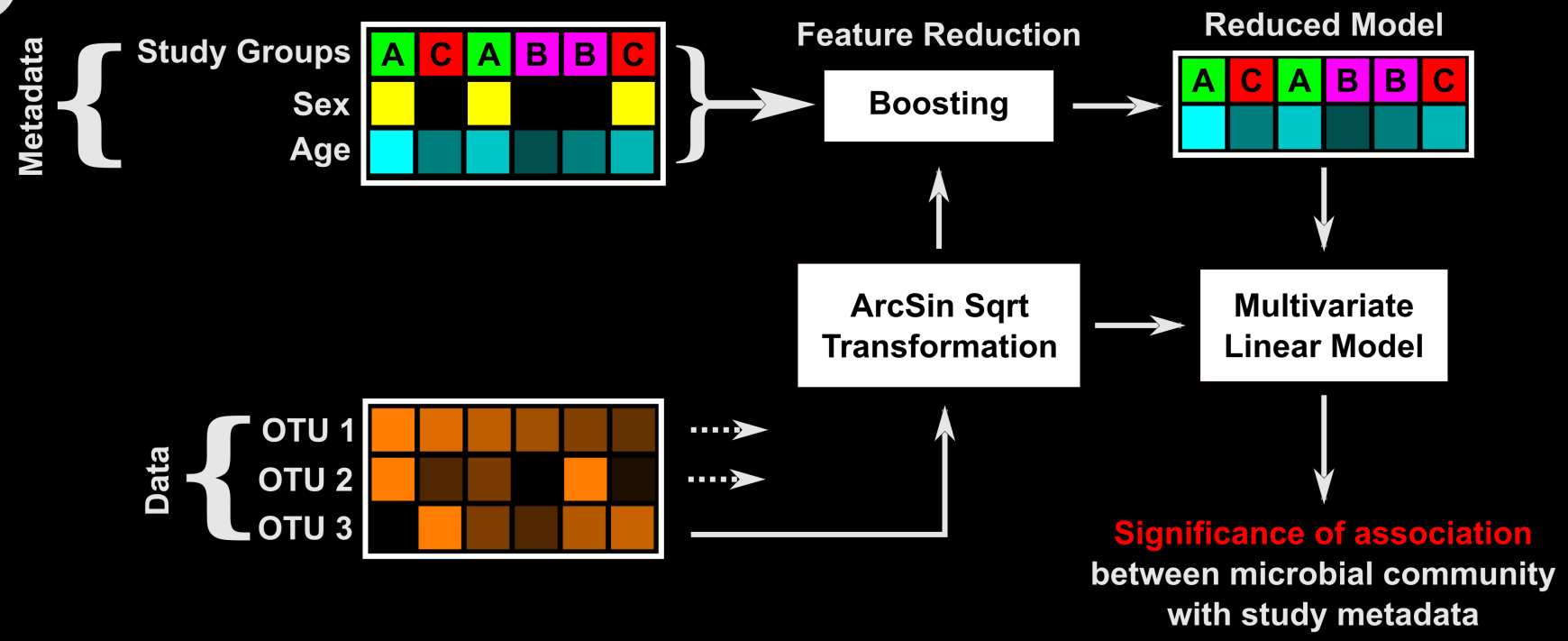
Properties of microbiome data

- Compositional nature (Σ = 1)
  - Abundance is relative, not absolute
- High dynamic range
- Often sparse (sample dominated by a few species)
- Noisy
- Hierarchical organization

Site	Oral	Gut	Oral	Gut	Oral	Gut
Clade1	0.40	0.87	0.43	0.68	0.47	0.32
Clade1|Bug1	0.40	0.56	0.07	0.31	0.42	0.27
Clade1|Bug2	0.00	0.30	0.36	0.37	0.04	0.05
Clade2	0.60	0.13	0.57	0.32	0.53	0.68
Clade2|Bug3	0.11	0.00	0.10	0.32	0.15	0.23
Clade2|Bug4	0.49	0.13	0.47	0.00	0.39	0.45

MaAsLin

Multivariate microbial Association with Linear models



- A more general solution for finding significant metagenomic associations in metadata-rich studies

Tim Tickle

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

Linking host and microbial function in ileal pouch inflammation

With Mark Silverberg

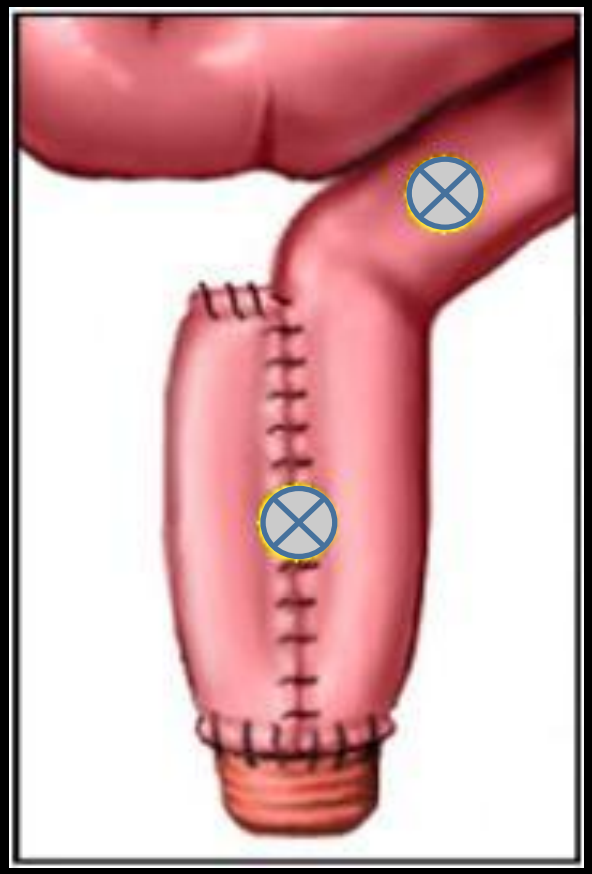
184 subjects with j-pouches at Mt. Sinai since 1981


Boyko Kabakchiev


Andrea Tyler


Xochi Morgan


Levi Waldron



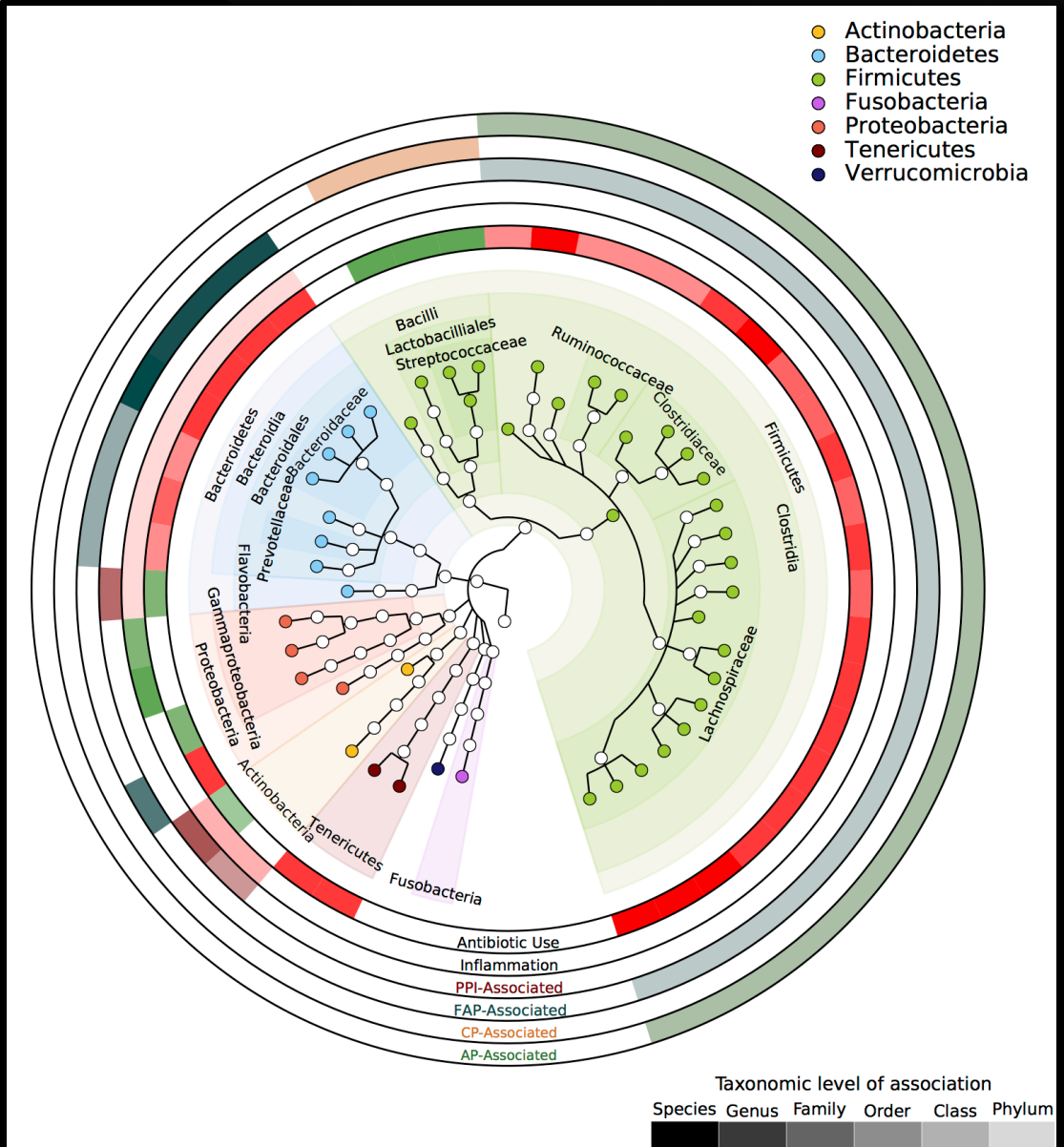
- **UC**
  - **NP** No Pouchitis
  - **(A/C)P** Pouchitis (Acute/Chronic)
  - **CDL** Crohn's Disease-Like Phenotype
- **FAP**
  - **FAP** Familial Adenomatous Polyposis

230 biopsies with host gene expression + microbiome

	FAP	NP	(A/C)P	CDL
Pouch	N 16	N 15	I 11	N/I 16
Pre-pouch ileum (PPI)	N 18	N 48	N 83	I 23

Morgan Genome Bio 2015

Multivariate association of microbes with pouchitis phenotypes

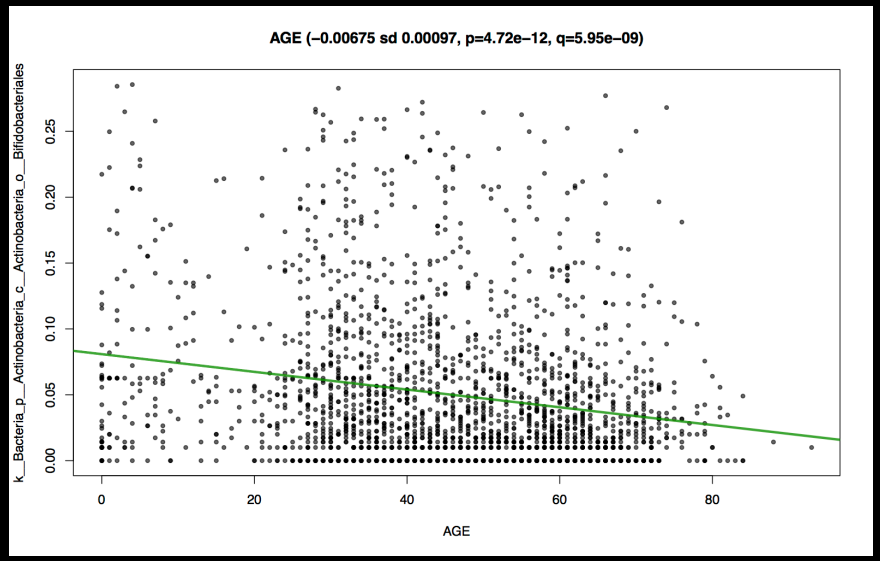


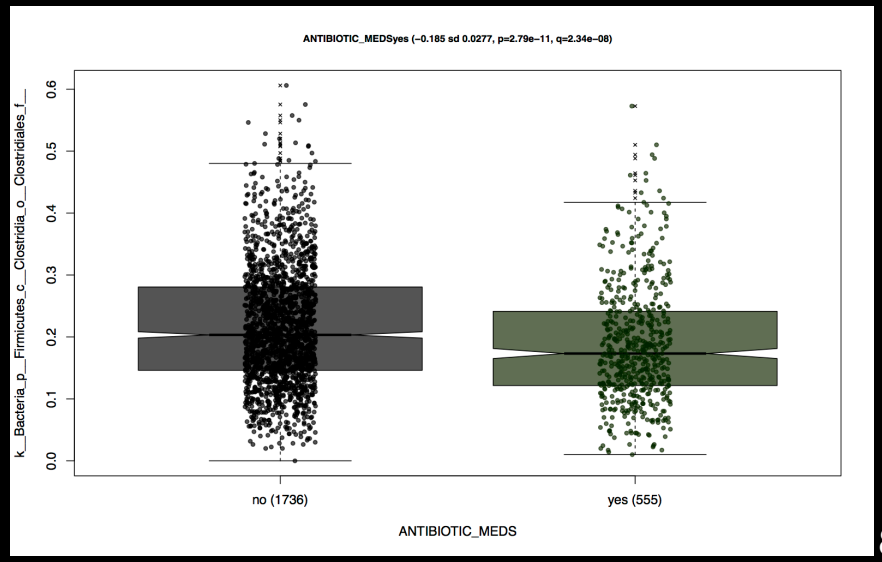

```
clade ~
    transcript +
    location +
    antibiotics +
    inflammation +
    phenotype
```

Can also include random effects (i.e. multiple samples per subject over time / space) and high dimensional models (e.g. genetics)

Multivariate association of microbes with phenotype in the American Gut

```
clade ~ acne_meds + age + alcohol + abx + asthma +
   BMI + carb% + country_now + country_birth +
   csection + diabetes + diet_type + dog + fat% +
   fiber + gluten + ibd + lactose_int + pregnant +
   protein% + race + sex
```



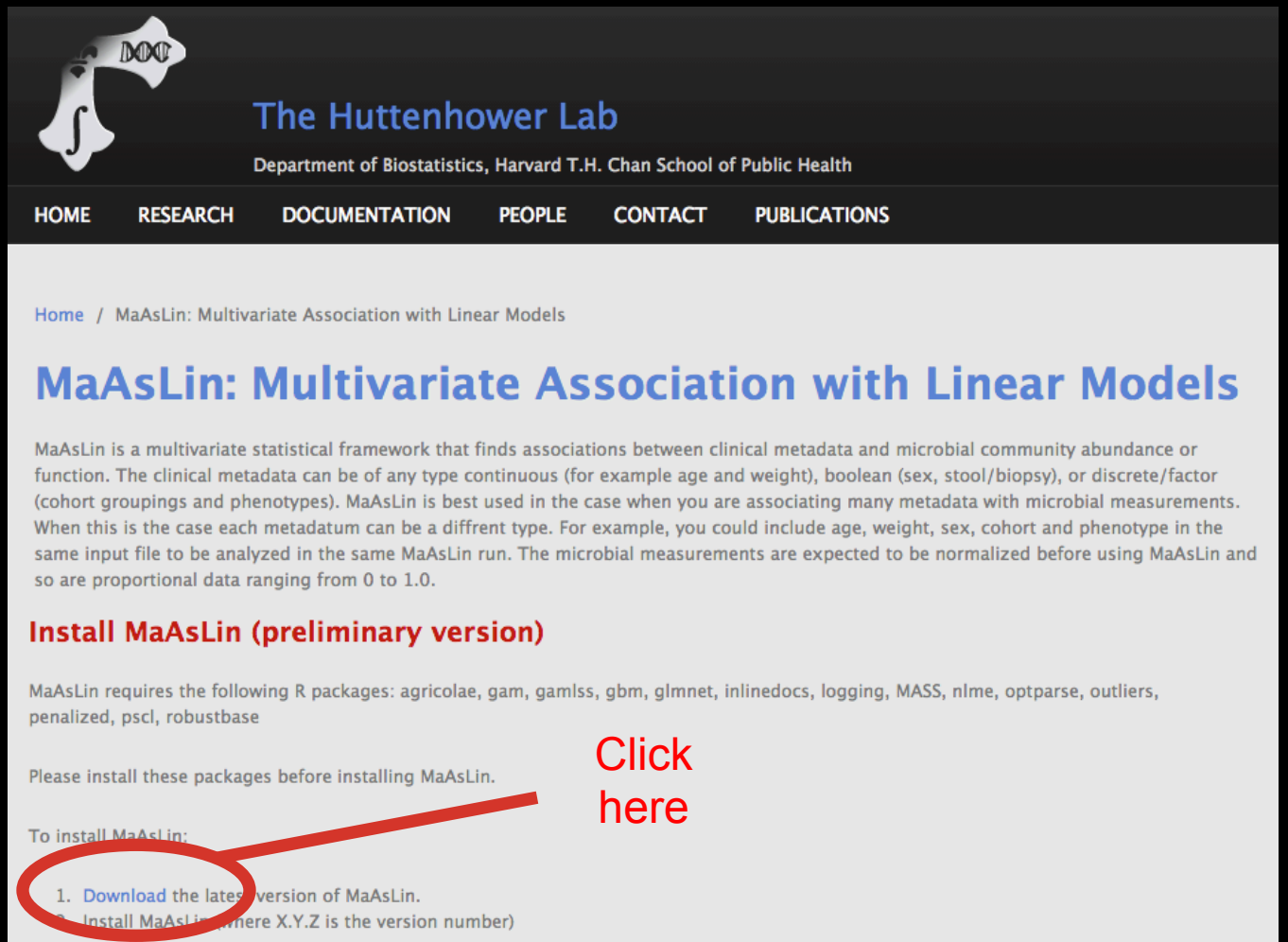


Setup notes reminder

- 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`**.

Multivariate associations: MaAsLin

- You can find the MaAsLin install and documentation at: http://huttenhower.sph.harvard.edu/maaslin



Multivariate associations: MaAsLin

- But we've already installed it! Take a look:

```
ln -s /home/ubuntu/metagenomics/maaslin/
./maaslin/R/Maaslin.R -h | less -S
```

3. screen (ssh)

```
Usage: ./maaslin/R/Maaslin.R [options] <data.tsv> <outputdir>


Options:
        -h, --help
                Show this help message and exit

        -i DATA.READ.CONFIG, --input_config=DATA.READ.CONFIG
                Optional configuration file describing data input format.

        -I DATA.R, --input_process=DATA.R
                Optional configuration script normalizing or processing data.

        -d SIGNIFICANCE, --fdr=SIGNIFICANCE
                The threshold to use for significance for the generated q-values
 (BH FDR). Anything equal to or lower than this is significant.  [Default 0.25]

        -r MINRELATIVEABUNDANCE, --minRelativeAbundance=MINRELATIVEABUNDANCE
                The minimum relative abundance allowed in the data. Values below
 this are removed and imputed as the median of the sample data.  [Default 1e-04]

        -p MINPREVALENCE, --minPrevalence=MINPREVALENCE
                The minimum percentage of samples in which a feature must have t
he minimum relative abundance in order not to be removed. Also this is the maxim
:
```

Starting simple: a univariate test with MaAsLin

- Let's start by associating one covariate with microbiome data :

```
/home/ubuntu/metagenomics/util/metadata.py \
    /home/ubuntu/metagenomics/data/hmp_metadata.dat \
    < /home/ubuntu/metagenomics/data/HMP.ab.filtered.txt \
    > HMP.ab.filtered.metadata.pcl
grep -E '^(sid)|(STSite)|(k__Bacteria)' HMP.ab.filtered.metadata.pcl \
    > HMP.ab.filtered.stsite.pcl
less -S HMP.ab.filtered.stsite.pcl
```

3. screen (ssh)

```
sid SRS043001   SRS017127   SRS021473   SRS011134   SRS050184   SRS011529   SRS0
STSite  Stool   Buccal_mucosa   Buccal_mucosa   Stool   Posterior_fornix   Stoo
k__Bacteria|p__Proteobacteria|c__Betaproteobacteria|o__Burkholderiales|f__Sutter
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Coriobacteriales|f__Coriobact
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Porphyromonadace
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae|g__R
k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Lachnospiraceae|g__B
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Actinomycetales|f__Micrococca
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__S
k__Bacteria|p__Proteobacteria|c__Epsilonproteobacteria  0.0 0.0049  1.10502 0.0
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Streptococcaceae|g__S
k__Bacteria|p__Fusobacteria|c__Fusobacteria|o__Leptotrichales|f__Leptotrichiacea
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Bacteroidaceae|g
k__Bacteria|p__Verrucomicrobia|c__Verrucomicrobiae|o__Verrucomicrobiales|f__Verr
k__Bacteria|p__Fusobacteria|c__Fusobacteria|o__Leptotrichales|f__Leptotrichiacea
k__Bacteria|p__Bacteroidetes|c__Bacteroidia 84.60804    0.17674 1.20942 58.53925
k__Bacteria|p__Proteobacteria|c__Epsilonproteobacteria|o__Campylobacterales 0.0
k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales  0.0 3.7702  5.24454 0.0 0.0
k__Bacteria|p__Firmicutes|c__Bacilli|o__Lactobacillales|f__Lactobacillaceae|g__L
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Porphyromonadace
k__Bacteria|p__Bacteroidetes|c__Bacteroidia|o__Bacteroidales|f__Porphyromonadace
k__Bacteria|p__Firmicutes|c__Negativicutes|o__Selenomonadales|f__Acidaminococcac
k__Bacteria|p__Actinobacteria|c__Actinobacteria|o__Bifidobacteriales|f__Bifidoba
HMP.ab.filtered.stsite.pcl
```

Starting simple: a univariate test with MaAsLin

- To run MaAsLin easily on one covariate:

```
./maaslin/R/Maaslin.R HMP.ab.filtered.stsite.pcl \
    HMP.ab.filtered.stsite --lastMetadata=2
```

3. screen (ssh)

```
 2015-08-13 13:31:44 INFO:maaslin:SRS054590
2015-08-13 13:31:44 INFO:maaslin:Removing data 42 for being all NA after QC
2015-08-13 13:31:45 INFO:maaslin:Removing the following for having only NAs afte
r cleaning (maybe due to only having NA after outlier testing).
2015-08-13 13:31:45 INFO:maaslin:k__Bacteria
2015-08-13 13:31:45 INFO:maaslin:Outputting to: HMP.ab.filtered.stsite/HMP.ab.fi
ltered_log.txt
2015-08-13 13:31:46 INFO:maaslin:Taxon 10/182
2015-08-13 13:31:49 INFO:maaslin:Taxon 20/182
2015-08-13 13:31:52 INFO:maaslin:Taxon 30/182
2015-08-13 13:31:54 INFO:maaslin:Taxon 40/182
2015-08-13 13:31:59 INFO:maaslin:Taxon 60/182
2015-08-13 13:32:02 INFO:maaslin:Taxon 70/182
2015-08-13 13:32:05 INFO:maaslin:Taxon 80/182
2015-08-13 13:32:08 INFO:maaslin:Taxon 90/182
2015-08-13 13:32:11 INFO:maaslin:Taxon 100/182
2015-08-13 13:32:13 INFO:maaslin:Taxon 110/182
2015-08-13 13:32:16 INFO:maaslin:Taxon 120/182
2015-08-13 13:32:19 INFO:maaslin:Taxon 130/182
2015-08-13 13:32:22 INFO:maaslin:Taxon 140/182
2015-08-13 13:32:24 INFO:maaslin:Taxon 150/182
2015-08-13 13:32:28 INFO:maaslin:Taxon 160/182
2015-08-13 13:32:30 INFO:maaslin:Taxon 170/182
2015-08-13 13:32:34 INFO:maaslin:Taxon 180/182
[chuttenhower@class03 ~]$
```

Starting simple: a univariate test with MaAsLin

- What are all these files!?!

```
ls -R HMP.ab.filtered.stsite
```

```
3. screen (ssh)
[chuttenhower@class03 ~]$ ls -R HMP.ab.filtered.stsite
HMP.ab.filtered.stsite:
generated_config            HMP.ab.filtered.stsite.tsv  QC
HMP.ab.filtered_log.txt     HMP.ab.filtered.stsite.txt
HMP.ab.filtered-STSite.pdf  HMP.ab.filtered-STSite.txt

HMP.ab.filtered.stsite/QC:
data.read.config      ProcessQC.txt              read-Merged.tsv
data.tsv              read_cleaned.read.config   Run_Parameters.txt
metadata.read.config  read_cleaned.tsv
metadata.tsv          read-Merged.read.config
[chuttenhower@class03 ~]$
```

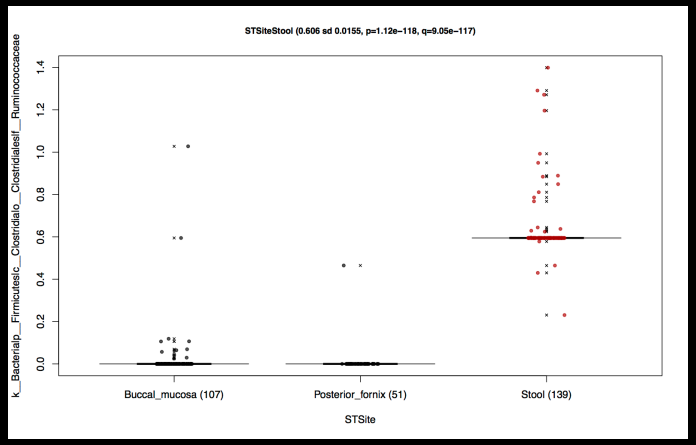
- First, processed inputs:
  - **`generated_config`** indicates how MaAsLin read your data
    - One matrix containing metadata model variables, one containing data
  - **`QC`** directory contains separate files for data + metadata
  - **`Run_Parameters.txt`** contains model variables
    - Was it sparse, were there random variables, what filtering criteria, etc.

Starting simple: a univariate test with MaAsLin

- Next, what you care about, outputs:
  - **`HMP.ab.filtered-STSite.txt`** lists associations between clades and the model variable STSite and their significance

Variable	Feature	Value	Coefficient	N	N not 0	P-value	Q-value
STSite	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillales_uncl|g__Gemella|s__Gemella_haemolysans	STSiteStool	-0.7036054	297	112	2.48E-196	1.60E-193
STSite	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillales_uncl|g__Gemella	STSiteStool	-0.6315029	297	114	1.04E-169	2.23E-167
STSite	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillales_uncl	STSiteStool	-0.6315029	297	114	1.04E-169	2.23E-167
STSite	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillales_uncl|g__Gemella|s__Gemella_haemolysans	STSitePosterior_fornix	-0.7047368	297	112	5.18E-163	8.35E-161
STSite	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillales_uncl|g__Gemella	STSitePosterior_fornix	-0.6344187	297	114	7.57E-138	8.12E-136
STSite	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales|f__Bacillales_uncl	STSitePosterior_fornix	-0.6344187	297	114	7.57E-138	8.12E-136
STSite	k__Bacteria|p__Firmicutes|c__Bacilli|o__Bacillales	STSiteStool	-0.5226617	297	120	3.27E-119	3.01E-117
STSite	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae	STSiteStool	0.60575613	297	151	1.12E-118	9.05E-117
STSite	k__Bacteria|p__Firmicutes|c__Clostridia|o__Clostridiales|f__Ruminococcaceae|g__Faecalibacterium	STSiteStool	0.5194707	297	143	1.88E-111	1.35E-109

  - **`HMP.ab.filtered-STSite.pdf`** plots them



  - **`HMP.ab.filtered_log.txt`** logs all tests, significant or not

Multivariate tests with MaAsLin

- Let's run a more interesting IBD model:

```
ln -s /home/ubuntu/metagenomics/data/ibd2012.pcl
less -S ibd2012.pcl
```

3. screen (ssh)

```
sample  7007    7010    7016    7018    7021    7022    7035    7037    7039
age 28  41  36  30  39  34  45  32  50  54  38  56  40  44  53  49  35  55  49
antibiotics 0   0   0   0   0   1   0   0   0   0   0   0   0   0   1   0   0
dx  CD  CD  UC  UC  CD  CD  CD  CD  CD  UC  CD  CD  CD  CD  CD  CD  CD  CD  CD
gender  0   1   0   0   0   0   0   1   1   0   1   0   1   1   0   0   0   0
ileal   1   1   0   0   0   1   1   1   1   0   1   1   1   1   0   1   0   1
immunosup   1   1   1   0   1   0   1   1   0   0   1   1   1   1   0   1   1
mesalamine  0   0   0   0   0   0   0   1   1   1   0   1   0   0   1   1   0
smoker  1   1   1   0   0   0   1   0   0   1   1   0   1   2   0   0   1   1
steroids    0   0   1   0   0   0   0   0   0   0   0   0   0   0   0   0   0
stool   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0   0
Archaea|Euryarchaeota|Methanobacteria|Methanobacteriales|Methanobacteriaceae
Archaea|Euryarchaeota|Methanobacteria|Methanobacteriales|Methanobacteriaceae|Met
Archaea|Euryarchaeota|Methanobacteria|Methanobacteriales|Methanobacteriaceae|Met
Bacteria    0.976701    0.923429    1   0.972956    0.803437    0.696739    0.94
Bacteria|Actinobacteria|Actinobacteria  0   0.00457143  0   0.0197628   0.003209
Bacteria|Actinobacteria|Actinobacteria|Actinomycetales  0   0.00114286  0   0.00
Bacteria|Actinobacteria|Actinobacteria|Actinomycetales|Actinomycetaceae 0   0
Bacteria|Actinobacteria|Actinobacteria|Actinomycetales|Actinomycetaceae|Actinomy
Bacteria|Actinobacteria|Actinobacteria|Actinomycetales|Actinomycetaceae|Varibacu
Bacteria|Actinobacteria|Actinobacteria|Actinomycetales|Actinomycetaceae|unclassi
Bacteria|Actinobacteria|Actinobacteria|Actinomycetales|Brevibacteriaceae|Breviba
Bacteria|Actinobacteria|Actinobacteria|Actinomycetales|Corynebacteriaceae|Coryne
Bacteria|Actinobacteria|Actinobacteria|Actinomycetales|Dermabacteraceae 0   0
ibd2012.pcl
```

Multivariate tests with MaAsLin

- Running this isn't any harder than before (it just takes a little longer):

```
./maaslin/R/Maaslin.R ibd2012.pcl ibd2012 --lastMetadata=11
```

3. screen (ssh)

```
2015-08-13 16:31:29 INFO:maaslin:Grubbs Test::Removing 6 outliers from unclassif
ied
2015-08-13 16:31:29 INFO:maaslin:7049
 2015-08-13 16:31:29 INFO:maaslin:7059
 2015-08-13 16:31:29 INFO:maaslin:7095
 2015-08-13 16:31:29 INFO:maaslin:7125
 2015-08-13 16:31:29 INFO:maaslin:7233
 2015-08-13 16:31:29 INFO:maaslin:7871
2015-08-13 16:31:29 INFO:maaslin:Grubbs Test::Removing 2 outliers from smoker
2015-08-13 16:31:29 INFO:maaslin:7164
 2015-08-13 16:31:29 INFO:maaslin:7610
2015-08-13 16:31:29 INFO:maaslin:Grubbs Test::Removing 1 outliers from steroids
2015-08-13 16:31:29 INFO:maaslin:7871
2015-08-13 16:31:30 INFO:maaslin:Outputting to: ibd2012/ibd2012_log.txt
2015-08-13 16:31:38 INFO:maaslin:Taxon 70/360
2015-08-13 16:31:42 INFO:maaslin:Taxon 100/360
2015-08-13 16:31:51 INFO:maaslin:Taxon 150/360
2015-08-13 16:31:54 INFO:maaslin:Taxon 170/360
2015-08-13 16:31:57 INFO:maaslin:Taxon 180/360
2015-08-13 16:31:59 INFO:maaslin:Taxon 190/360
2015-08-13 16:32:04 INFO:maaslin:Taxon 210/360
2015-08-13 16:32:06 INFO:maaslin:Taxon 220/360
2015-08-13 16:32:10 INFO:maaslin:Taxon 260/360
2015-08-13 16:32:19 INFO:maaslin:Taxon 360/360
[chuttenhower@class03 ~]$
```

Multivariate tests with MaAsLin

- We can see all of the significant results:

```
less -S ibd2012/ibd2012.txt
```

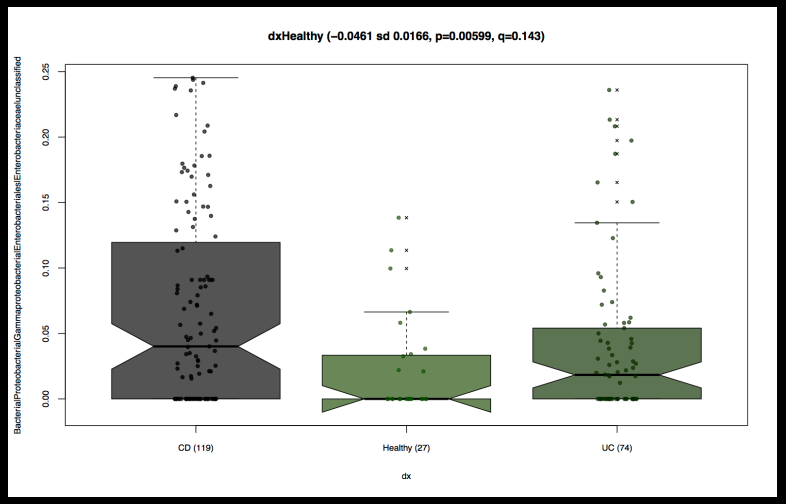
3. screen (ssh)

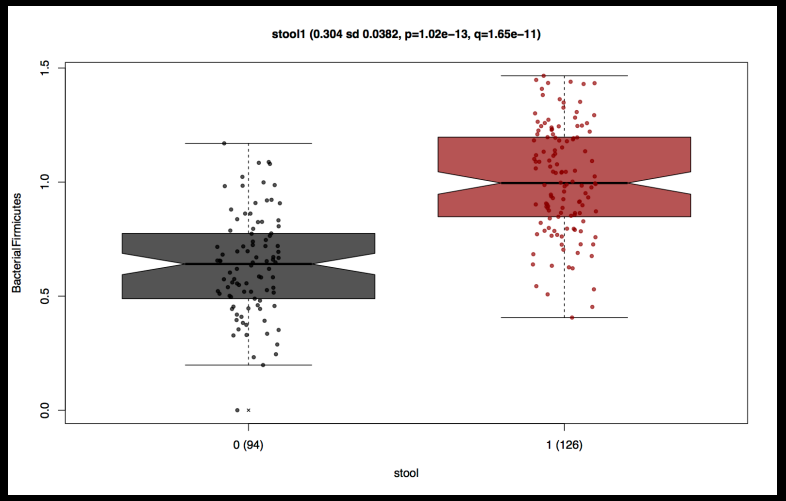
```
   Variable    Feature Value   Coefficient N   N.not.0 P.value Q.value
1  age Bacteria|Bacteroidetes  age 0.00434507175397982 220 212 0.00016448840082
2  age Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales|Bacteroidaceae|Bacteroi
3  age Bacteria|Firmicutes|Bacilli|Lactobacillales|Leuconostocaceae|Leuconostoc
4  age Bacteria|Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Sporobacter
5  age Bacteria|Actinobacteria|Actinobacteria|Bifidobacteriales|Bifidobacteriac
6  age Bacteria|Actinobacteria|Actinobacteria|Bifidobacteriales|Bifidobacteriac
7  age Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales    age 0.00409312520323
8  age Bacteria|Firmicutes|Bacilli|Lactobacillales|Leuconostocaceae    age -0.0
9  age Bacteria|Firmicutes age -0.00286276397618523    220 219 0.00333716992643
10 age Bacteria|Proteobacteria|Gammaproteobacteria|Pasteurellales|Pasteurellace
11 age Bacteria|Firmicutes|Clostridia|Clostridiales|Incertae_Sedis_XI|Parvimona
12 age Bacteria|Firmicutes|Clostridia|unclassified age 0.000111560534782389
13 age Bacteria|Bacteroidetes|Bacteroidia|Bacteroid
14 age Bacteria|Bacteroidetes|Bacteroidia|Bacteroi
15 age Bacteria|Firmicutes|Clostridia|Clostridiales
16 age Bacteria|Firmicutes|Clostridia|Clostridiales
17 antibiotics Bacteria|Actinobacteria|Actinobacter
18 antibiotics Bacteria|Firmicutes|Clostridia|Clost
19 antibiotics Bacteria|Bacteroidetes|Bacteroidia|B
20 antibiotics Bacteria|Bacteroidetes  antibiotics1
21 antibiotics Bacteria|Bacteroidetes|Bacteroidia|B
22 antibiotics Bacteria|Bacteroidetes|Bacteroidia|B
23 antibiotics Bacteria|Bacteroidetes|Bacteroidia|B
ibd2012/ibd2012.txt
```

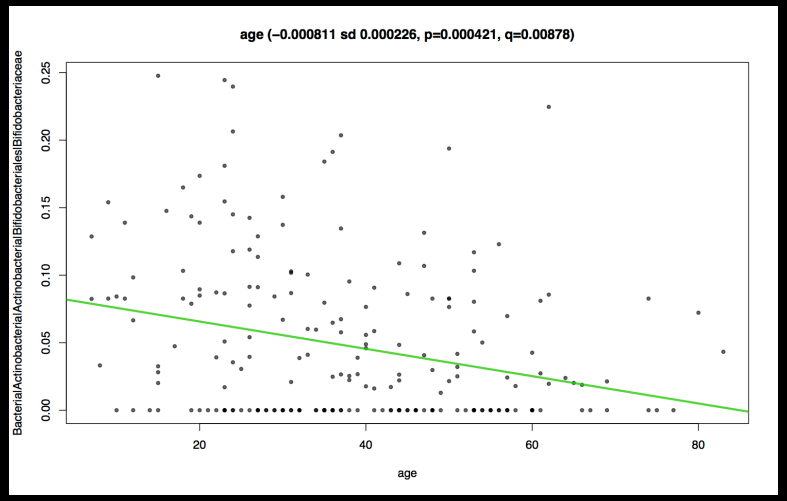
Variable	Feature	Value	Coefficient	N	N.not.0	P.value	Q.value
age	Bacteria|Bacteroidetes	age	0.00434507	220	212	0.00016449	0.00442063
age	Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales|Bacteroidaceae|Bacteroides	age	0.00387834	220	206	0.00033318	0.00781462
age	Bacteria|Firmicutes|Bacilli|Lactobacillales|Leuconostocaceae|Leuconostoc	age	-0.000176	220	29	0.00035114	0.00808887
age	Bacteria|Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Sporobacter	age	0.00014751	220	53	0.0003815	0.0084851
age	Bacteria|Actinobacteria|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae	age	-0.0008113	220	126	0.00042121	0.00877677
age	Bacteria|Actinobacteria|Actinobacteria|Bifidobacteriales|Bifidobacteriaceae|Bifidobacterium	age	-0.0008086	220	126	0.00042663	0.00877677
age	Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales	age	0.00409313	220	212	0.00083297	0.01557283
age	Bacteria|Firmicutes|Bacilli|Lactobacillales|Leuconostocaceae	age	-0.0001888	220	39	0.00089099	0.01618847
age	Bacteria|Firmicutes	age	-0.0028628	220	219	0.00333717	0.05204288
age	Bacteria|Proteobacteria|Gammaproteobacteria|Pasteurellales|Pasteurellaceae|Actinobacillus	age	-0.0001436	220	27	0.00362747	0.05570764
age	Bacteria|Firmicutes|Clostridia|Clostridiales|Incertae_Sedis_XI|Parvimonas	age	0.00016739	220	26	0.00704009	0.09460119
age	Bacteria|Firmicutes|Clostridia|unclassified	age	0.00011156	220	47	0.0082163	0.10815338
age	Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales|Porphyromonadaceae|Parabacteroides	age	0.00097951	220	147	0.00922914	0.11672154
age	Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales|Porphyromonadaceae	age	0.00101908	220	163	0.00942202	0.11800397
age	Bacteria|Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Butyricicoccus	age	0.00036856	220	171	0.01802349	0.19215128
age	Bacteria|Firmicutes|Clostridia|Clostridiales|Veillonellaceae|unclassified	age	0.00022306	220	104	0.02138539	0.21714042
antibiotics	Bacteria|Actinobacteria|Actinobacteria|Coriobacteriales|Coriobacteriaceae|Collinsella	antibiotics1	-0.0862293	220	118	5.82E-06	0.00025891
antibiotics	Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Dorea	antibiotics1	-0.0734784	220	197	4.79E-05	0.00162547
antibiotics	Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales|Rikenellaceae	antibiotics1	-0.0428438	220	132	0.01213827	0.14365478
antibiotics	Bacteria|Bacteroidetes	antibiotics1	-0.1419265	220	212	0.01485215	0.16660234
antibiotics	Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales|Rikenellaceae|Alistipes	antibiotics1	-0.0402089	220	132	0.01502882	0.16713084
antibiotics	Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales	antibiotics1	-0.1415995	220	212	0.01525422	0.16818757
antibiotics	Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales|Porphyromonadaceae|Odoribacter	antibiotics1	-0.0129536	220	72	0.01542788	0.16866076
antibiotics	Bacteria|Firmicutes|Clostridia|Clostridiales|Veillonellaceae|unclassified	antibiotics1	-0.0119452	220	104	0.01697982	0.18253305
antibiotics	Bacteria|Firmicutes|Clostridia|Clostridiales|Veillonellaceae|Phascolarctobacterium	antibiotics1	-0.0278794	220	95	0.02117856	0.21682815
antibiotics	Bacteria|Bacteroidetes|Bacteroidia|Bacteroidales|Bacteroidaceae|Bacteroides	antibiotics1	-0.1250394	220	206	0.02171404	0.21714042
dx	Bacteria|Firmicutes|Clostridia|Clostridiales|Lachnospiraceae|Roseburia	dxHealthy	0.12959193	220	177	2.55E-07	2.63E-05
dx	Bacteria|Proteobacteria|Gammaproteobacteria|Enterobacteriales|Enterobacteriaceae|Cronobacter	dxHealthy	-0.0190217	220	66	3.08E-05	0.00193979
dx	Bacteria|Firmicutes|Clostridia|Clostridiales|Ruminococcaceae|Ruminococcus	dxHealthy	0.05645538	220	114	0.00038492	0.01460437

Multivariate tests with MaAsLin

- Or you can visualize raw data for individual variables:







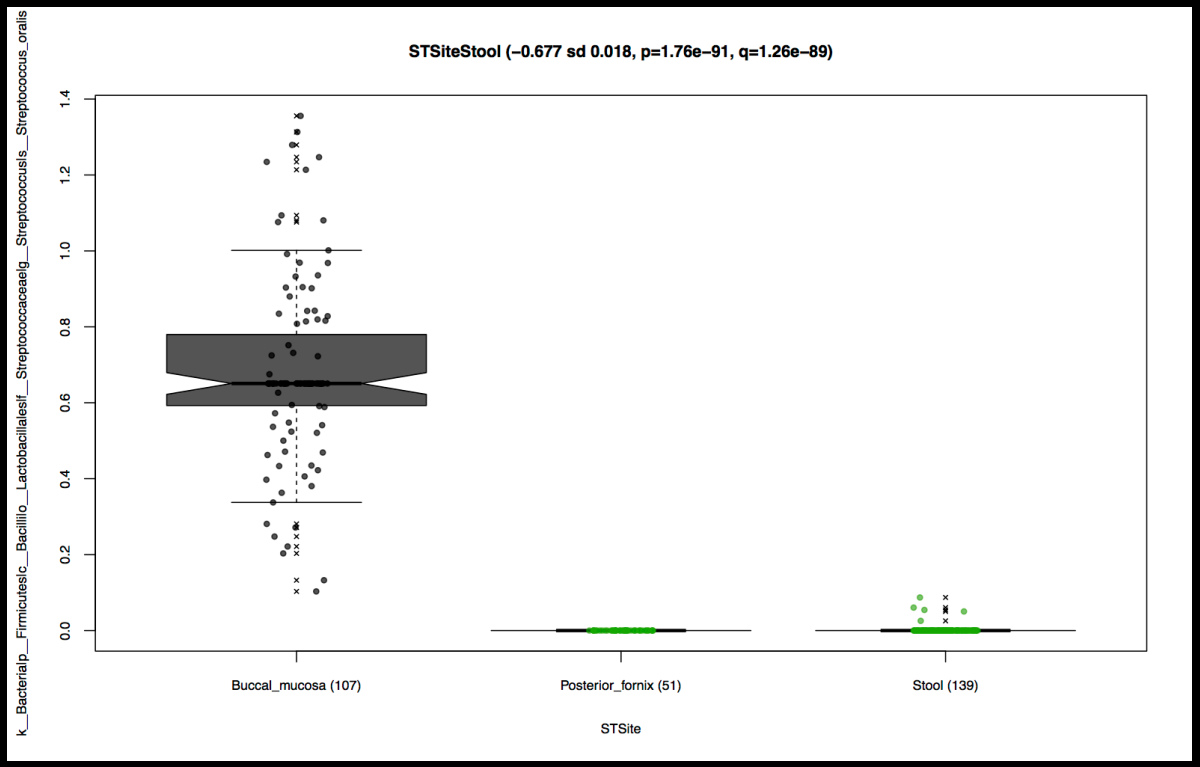
Advanced MaAsLin: random effects

- What if you have multiple samples / subject?

```
grep -E '^(sid)|(RANDSID)|(STSite)|(k__)' HMP.ab.filtered.metadata.pcl > HMP.ab.filtered.subject.pcl
./maaslin/R/Maaslin.R HMP.ab.filtered.subject.pcl HMP.ab.filtered.subject --lastMetadata=3 -R RANDSID
```

3. screen (ssh)

```
iteration 2
iteration 1
iteration 2
iteration 1
iteration 2
iteration 1
iteration 2
iteration 1
iteration 2
iteration 1
iteration 2
iteration 1
iteration 2
iteration 1
iteration 2
iteration 1
iteration 2
2015-08-13 16:53:53 INFO:maaslin:Taxon 180/183
iteration 1
iteration 2
iteration 1
iteration 2
iteration 1
iteration 2
iteration 1
iteration 2
[chuttenhower@class03 ~]$
```



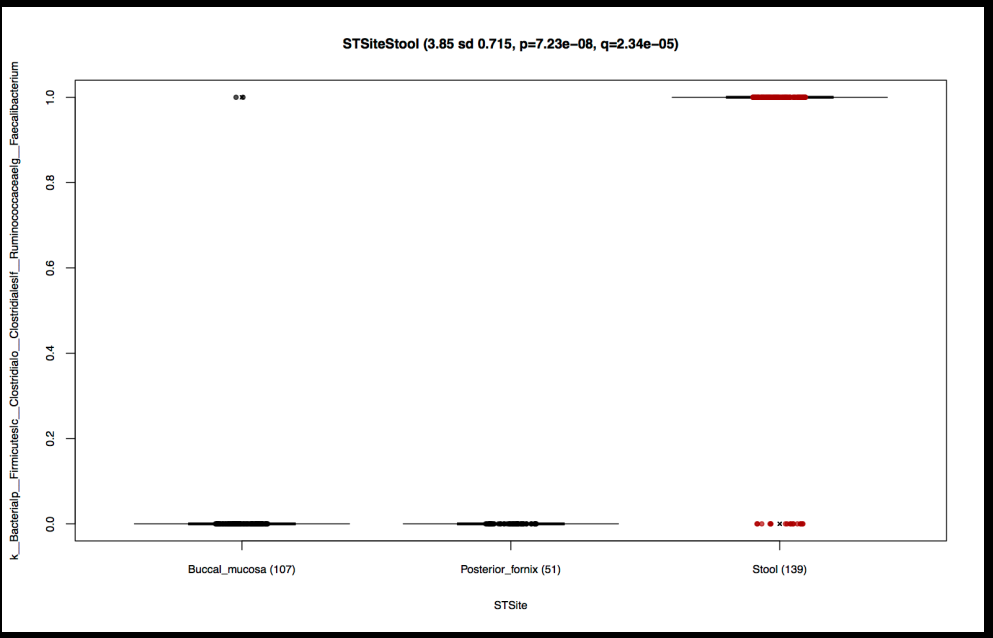
Advanced MaAsLin: other models

- What if you like to use a zero-inflated negative binomial?
  - Be warned, R's version is buggy as all get go...

```
./maaslin/R/Maaslin.R HMP.ab.filtered.stsite.pcl HMP.ab.filtered.negbin --lastMetadata=2 -z -m neg_binomial
```

3. screen (ssh)

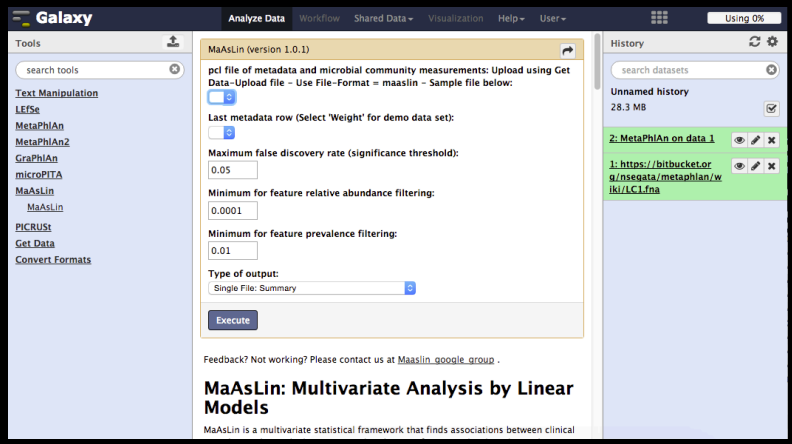
```
  system is computationally singular: reciprocal condition number = 3.70325e-22
Error in solve.default(as.matrix(fit$hessian)) :
  system is computationally singular: reciprocal condition number = 6.55509e-17
2015-08-13 16:54:58 INFO:maaslin:Taxon 140/182
Error in solve.default(as.matrix(fit$hessian)) :
  system is computationally singular: reciprocal condition number = 6.80142e-20
2015-08-13 16:55:00 INFO:maaslin:Taxon 150/182
Error in solve.default(as.matrix(fit$hessian)) :
  Lapack routine dgesv: system is exactly singular: U[7,7] = 0
Error in solve.default(as.matrix(fit$hessian)) :
  system is computationally singular: reciprocal condition number = 2.32384e-21
Error in solve.default(as.matrix(fit$hessian)) :
  system is computationally singular: reciprocal condition number = 1.06805e-43
Error in solve.default(as.matrix(fit$hessian)) :
  system is computationally singular: reciprocal condition number = 3.70325e-22
2015-08-13 16:55:06 INFO:maaslin:Taxon 160/182
2015-08-13 16:55:09 INFO:maaslin:Taxon 170/182
Error in solve.default(as.matrix(fit$hessian)) :
  system is computationally singular: reciprocal condition number = 2.56586e-21
Error in solve.default(as.matrix(fit$hessian)) :
  system is computationally singular: reciprocal condition number = 1.08876e-21
2015-08-13 16:55:14 INFO:maaslin:Taxon 180/182
Error in solve.default(as.matrix(fit$hessian)) :
  system is computationally singular: reciprocal condition number = 6.01582e-20
[chuttenhower@class03 ~]$
```



Advanced MaAsLin: more tricks

- MaAsLin can...
  - Change all QC and significance parameters
    - Minimum relative abundance, prevalence, FDR threshold, method, etc.
  - Skip some metadata
    - Check out the `read.config` file documentation and `-i` flag
  - Use other regularization approaches
    - Not just boosting: LASSO, forward, backward, none, etc. (`-s` flag)
  - Test all-against-all features, e.g. all bugs against all genetic variants
    - `-a` flag, use with caution – power!

- And there's a beta Galaxy interface
  - http://huttenhower.sph.harvard.edu/maaslin



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:

bioBakery

Virtual environment for Huttenhower tools

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:

- **HUMAnN** • Microbial species and associated genes and pathways
- **MetaPhlAn** • Microbial species and abundances
- **PhyloPhlAn** • Reconstruction of phylogenetic trees
- **PICRUSt** • Predict metagenome functional content from marker gene
- **ShortBRED** • Abundance of proteins of interest in genetic data

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:

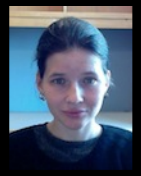
- **ARepA** • Extract 'omics data from repositories
- **CCREPE** • Assess the significance of general similarity measures in compositional datasets
- **LEfSe** • Association between metadata (max. 2) and microbial species and abundances
- **MaAsLin** • Association between metadata (no restriction) and microbial species and abundances
- **microPITA** • Sample selection in two stage-tiered studies

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:

Thank you!


Curtis Huttenhower


Xochitl Morgan


Minah Iqbal


Lauren McIver


George Weingart

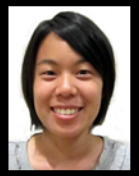

Aleksandar Kostic


Gholamali Rahnavard


Melanie Schirmer


Alexandra Sirota-Madi


Ayshwarya Subramanian


Tiffany Hsu


Boyu Ren


Emma Schwager

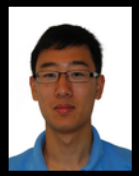

Koji Yasuda


Siyuan Ma


Casey DuLong


Jim Kaminski


Randall Schwager


Andy Shi


Moran Yassour


Luc Bijnens


Tommi Vatanen


Levi Waldron


Nicola Segata

Wendy Garrett
Michelle Rooks

Ruth Ley
Omry Koren

Dirk Gevers
Kat Huang

Ramnik Xavier
Harry Sokol
Dan Knights
Moran Yassour

Rob Beiko
Morgan Langille

Rob Knight
Greg Caporaso
Jesse Zaneveld

Mark Silverberg
Boyko Kabakchiev
Andrea Tyler

Jacques Izard
Katherine Lemon

Bruce Sands

Human Microbiome Project

Owen White
Joe Petrosino
George Weinstock
Karen Nelson
Lita Proctor
Sahar Abubucker
Brandi Cantarel
Alyx Schubert
Mathangi Thiagarajan
Beltran Rodriguez-Mueller
Erica Sodergren
Anthony Fodor
Marty Blaser
Jacques Ravel
Pat Schloss
Makedonka Mitreva
Yuzhen Ye
Mihai Pop
Larry Forney
Barbara Methe

Bruce Birren
Mark Daly
Doyle Ward
Ashlee Earl