humann2_metaphlann2_lefse_picrust

November 16, 2017

humann2 is a tool for analyzing whole-genome shotgun sequencing data

```
In [2]: # install humann2 by running pip install humann2
        # already done by Vijay
```

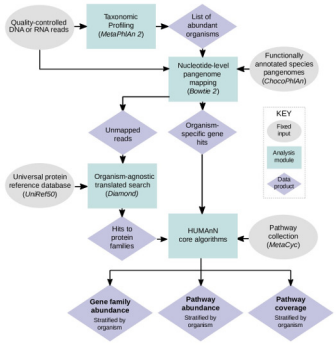
- neocleotide database is called chocophlan
- protein database is called uniref

```
In [ ]: # download databases
        humann2_databases --download chocophlan DEMO humann2_database_downloads
        humann2_databases --download uniref DEMO_diamond humann2_database_downloads
```

- humann2 metagenomics workflow

```
In [4]: from IPython.display import Image
        Image(filename = "data/humann2_diamond_500x500.jpg", width=500)
```

Out[4]:



- let's do a demo run
- directly compare demo.fastq to the protein database
- skip nucleotide search

```
In [1]: humann2 --input data/demo.fastq \
                --output out_humann2 \
                --bypass-nucleotide-search
```

```
Creating output directory: /Users/husenzhang/Documents/GitHub/FAES_metagenomics/out
Output files will be written to: /Users/husenzhang/Documents/GitHub/FAES_metagenomi

Running diamond ...


Aligning to reference database: uniref90_demo_prots.dmnd

Total bugs after translated alignment: 1
unclassified: 988 hits

Total gene families after translated alignment: 47

Unaligned reads after translated alignment: 95.4238095238 %


Computing gene families ...

Computing pathways abundance and coverage ...

Output files created:
/Users/husenzhang/Documents/GitHub/FAES_metagenomics/out_humann2/demo_genefamilies.
/Users/husenzhang/Documents/GitHub/FAES_metagenomics/out_humann2/demo_pathabundance
/Users/husenzhang/Documents/GitHub/FAES_metagenomics/out_humann2/demo_pathcoverage.
```

- Gene family abundance

```
In [4]: head out_humann2/demo_genefamilies.tsv

# Gene Family        demo_Abundance-RPKs
UNMAPPED        20039.0000000000
UniRef90_X6L320         28.0905985144
UniRef90_X6L320|unclassified        28.0905985144
UniRef90_U5FT06         25.9926484436
UniRef90_U5FT06|unclassified        25.9926484436
UniRef90_W8YTG4         25.2752668904
UniRef90_W8YTG4|unclassified        25.2752668904
UniRef90_Q9ZUH4         23.5421011503
UniRef90_Q9ZUH4|unclassified        23.5421011503
```

- pathway abundance

```
In [6]: head out_humann2/demo_pathabundance.tsv

# Pathway         demo_Abundance
UNMAPPED        6418.1601776341
```

```
UNINTEGRATED        80.8363195902
UNINTEGRATED|unclassified        80.8363195902
PWY-6305: putrescine biosynthesis IV        30.3913024756
PWY-6305: putrescine biosynthesis IV|unclassified        30.3913024756
PWY-4203: volatile benzenoid biosynthesis I (ester formation)        22.5319052245
PWY-4203: volatile benzenoid biosynthesis I (ester formation)|unclassified        2
PWY490-3: nitrate reduction VI (assimilatory)        21.3761301200
PWY490-3: nitrate reduction VI (assimilatory)|unclassified        21.3761301200
```

- hmp_pathabund.pcl is a human microbiome dataset
- from bitbucket.org/biobakery/biobakery/raw/tip/demos/biobakery_demos/data/humann2/input/hmp
- we use this dataset as an example of plotting
- look at the data

```
In [3]: head -4 data/hmp_pathabund.pcl | cut -f1-4
```

```
FEATURE \ SAMPLE        SRS011084        SRS011086        SRS011090
STSite        Stool        Tongue_dorsum        Buccal_mucosa
1CMET2-PWY: N10-formyl-tetrahydrofolate biosynthesis        0.000498359        0.00
1CMET2-PWY: N10-formyl-tetrahydrofolate biosynthesis|g__Acidovorax.s__Acidovorax_eb
```

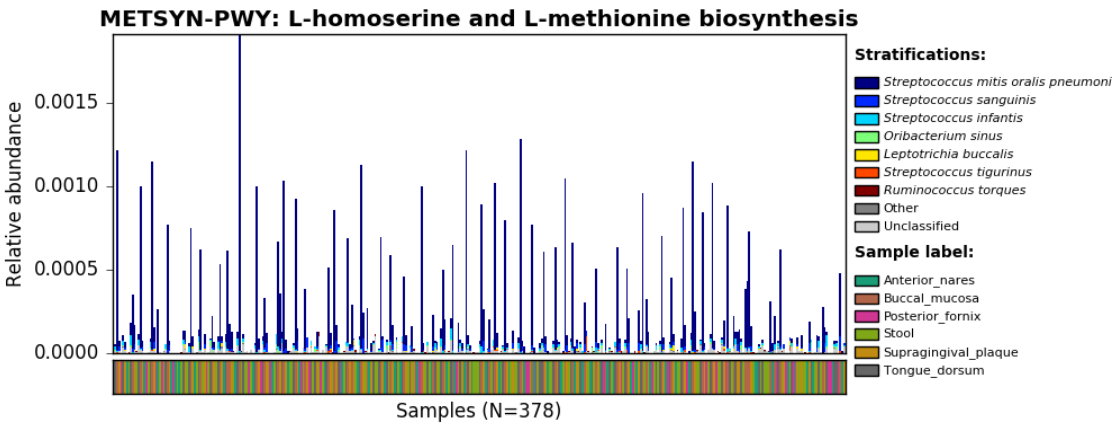
- now plot the data using humann2 barplot script
- this script depends on matplotlib

```
In [7]: humann2_barplot --input data/hmp_pathabund.pcl \
          --focal-feature METSYN-PWY \
          --focal-metadatum STSite \
          --last-metadatum STSite \
          --output out_humann2/plot1.png
```

- we produced a plot called plot1.png
- now take a look at it by double click it

```
In [14]: from IPython.display import Image
         PATH = "out_humann2/"
         Image(filename = PATH + "plot1.png", width=550)
```

```
Out[14]:
```

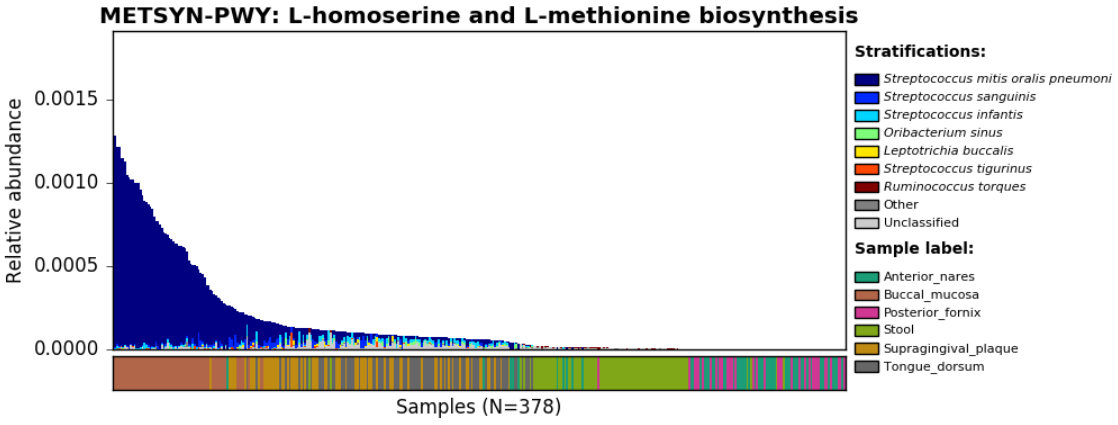


- the plot is messy
- let's "sort" on the stratified abundance:

```
In [4]: humann2_barplot --sort sum \
                --input data/hmp_pathabund.pcl \
                --focal-feature METSYN-PWY \
                --focal-metadatum STSite \
                --last-metadatum STSite \
                --output out_humann2/plot2.png
```

```
In [1]: from IPython.display import Image
        PATH = "out_humann2/"
        Image(filename = PATH + "plot2.png", width=550)
```

```
Out[1]:
```

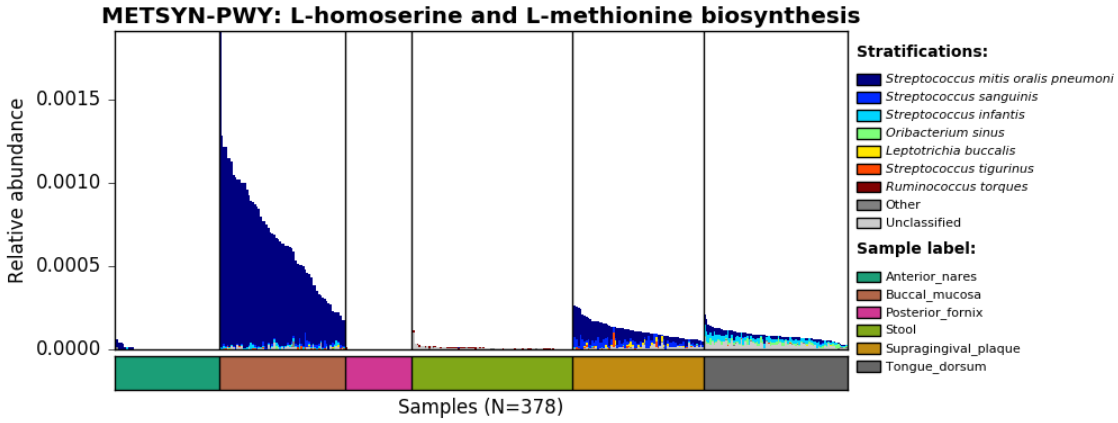


- A pattern has started to emerge: we can clearly see that the oral body sites are enriched on the left (high) end of the plot,
- We can continue this line of analysis with an additional grouping by body site:

```
In [1]: humann2_barplot --sort sum metadata \
        --input data/hmp_pathabund.pcl \
        --focal-feature METSYN-PWY \
        --focal-metadatum STSite \
        --last-metadatum STSite \
        --output out_humann2/plot3.png
```

```
In [2]: from IPython.display import Image
        PATH = "out_humann2/"
        Image(filename = PATH + "plot3.png", width=550)
```

Out[2]:

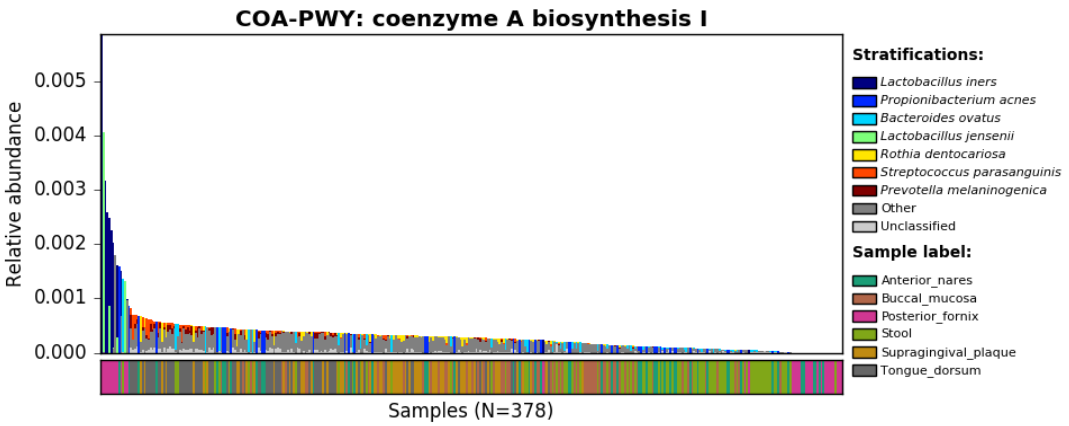


one additional pathway, COA-PWY: coenzyme A biosynthesis, which is more broadly conserved across body sites:

```
In [1]: humann2_barplot --sort sum --input data/hmp_pathabund.pcl \
        --focal-feature COA-PWY --focal-metadatum STSite \
        --last-metadatum STSite --output out_humann2/plot4.png
```

```
In [1]: from IPython.display import Image
        PATH = "out_humann2/"
        Image(filename = PATH + "plot4.png", width=550)
```

Out[1]:

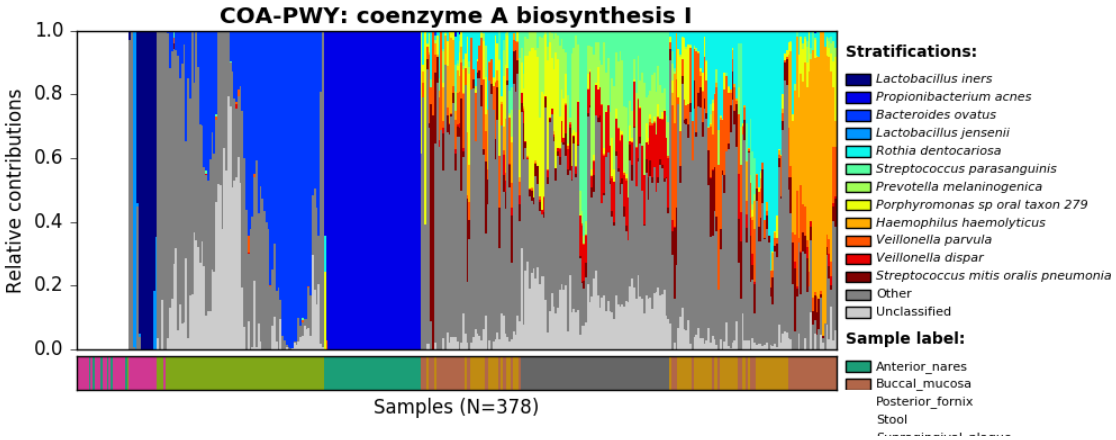


sorting by ecological similarity, normalizing pathway contributions within-sample, and expanding the list of species highlighted:

```
In [1]: humann2_barplot --sort similarity --top-strata 12 \
        --scaling normalize --input data/hmp_pathabund.pcl \
        --focal-feature COA-PWY --focal-metadatum STSite \
        --last-metadatum STSite --output out_humann2/plot5.png
```

```
In [5]: from IPython.display import Image
        PATH = "out_humann2/"
        Image(filename = PATH + "plot5.png", width=600)
```

Out[5]:



that's a wrap for humann2

```
In [ ]:
```