SATURDAY, November 7, 2020

Canada Future Directions in IBD

Microbiome Workshop

Part 1

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Why is it so difficult to precisely define mechanistic roles of microbes in health and disease, and to use microbiome data to predict disease?

- Individual **heterogeneity** in microbiome means it is difficult to separate healthy from patients (at the individual level)
- There are many **redundant** pathways that can be carried out by diverse bacteria (e.g. 4 pathways for butyrate synthesis, and dozen of species with at least one of these pathways
- Some activities are very **strain specific** (e.g. novel biochemical pathways)

See extra slides

Methodologies for Analysis the Microbiota

1. Marker Gene Profiling *e.g. 16S amplicon sequencing*
2. Shotgun Metagenomics
3. Metatranscriptomics
4. Culture and Culture-Enriched Metagenomics

Marker Gene Profiling *e.g. 16S amplicon sequencing*

Advantages

- Laboratory methods are robust and straightforward
- Inexpensive
- Bioinformatics are straightforward and do not require significant computation resources
- Work even for low biomass samples, or low microbial load in presence of high host – *with special precautions and extra controls*

Limitations

- Obtain only taxonomic information
- Variability based on the methods used
- Most standard approaches (variable region amplicon sequencing) do not resolve many taxa very well)

Marker Gene Profiling *e.g. 16S amplicon sequencing*

Limitations

- Obtain only taxonomic information

PICRUSt2: An improved and extensible approach for metagenome inference Nat Biotechnol. 2020
Douglas et al (senior author Morgan Langille )

- Updates version of the original program which includes expanded databases and gene families
- Allows for incorporating custom databases
- For prediction of core metabolic pathways does quite well

Shotgun Metagenomics

Advantages

- Laboratory methods are robust and straightforward
- Bioinformatics for basic analysis are available and most commonly used pipelines are
- Provides both taxonomic and functional information about the microbiome
- Can identify novel genes functions (depending on analysis pipelines)

Limitations

- Somewhat more expensive
- Common analysis tools (short read based) provide limited data
- More in-depth analysis much more complicated.
- Low microbial load in presence of high host means very little microbial data with out good methods to deplete host DNA

Metatranscriptomics

Advantages

- Laboratory methods are robust and straightforward
- Bi… …sed pi…
- Pr… m…
- Ca…

Metatranscriptomics - all of the same, provides information about what genes are being expressed

* *but with the caveat that bacterial transcription changes in 10s of seconds and bacterial mRNA half life is a few minutes. It is hard to process and stabilize samples fast enough…*

Limit…

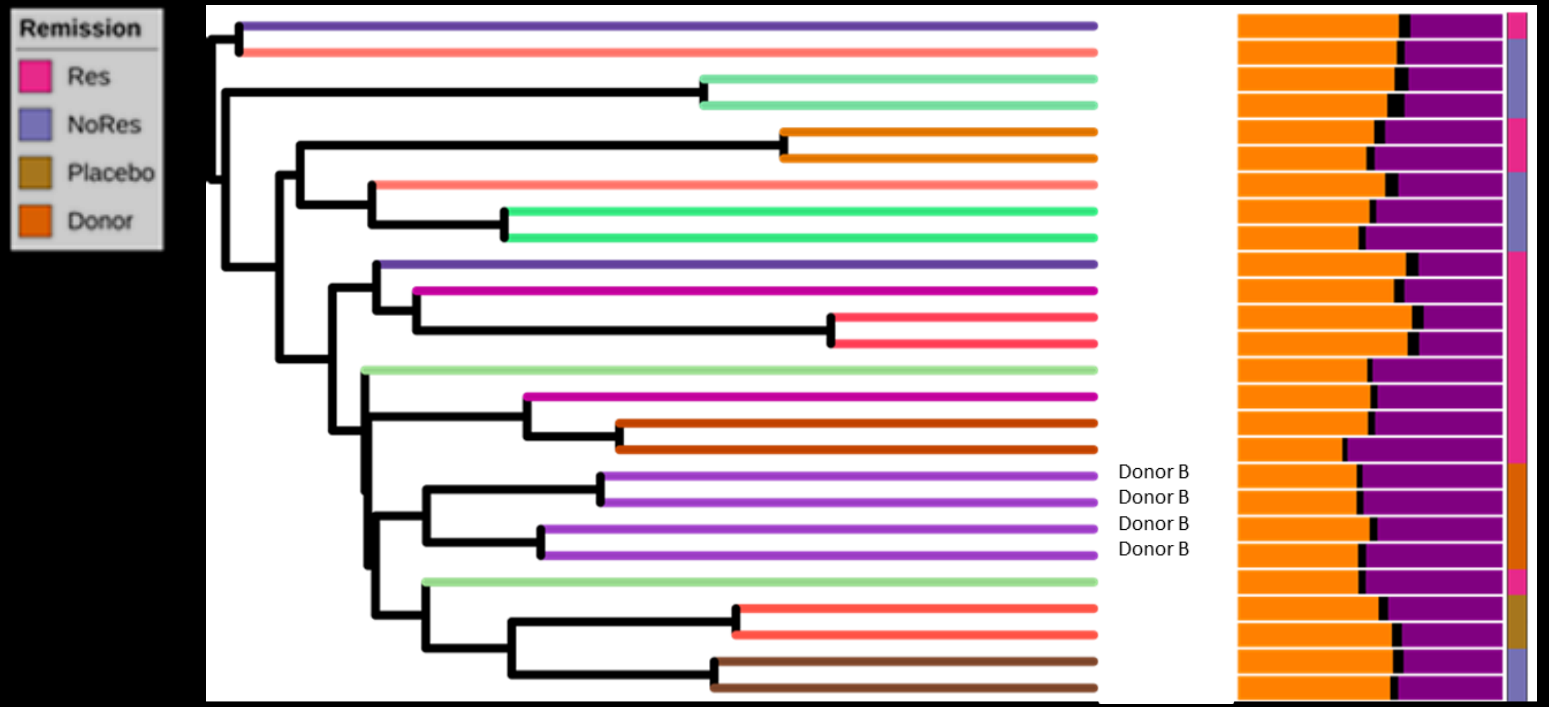
- So…
- Co…
- M…
- Low microbial load in presence of high host means very little microbial data with out good methods to deplete host DNA

Shotgun Metagenomics

Limitations

- Common analysis tools (short read based) provide limited data

Short read function analysis of metagenomic data - FMT Study in Ulcerative Colitis



Patients look most like themselves after FMT whether or not they were responders, they do not look like Donor B.

The microbiota cluster by function (same result as 16S profiling)

Sharok Shekarriz, unpublished

Shotgun Metagenomics

Limitations

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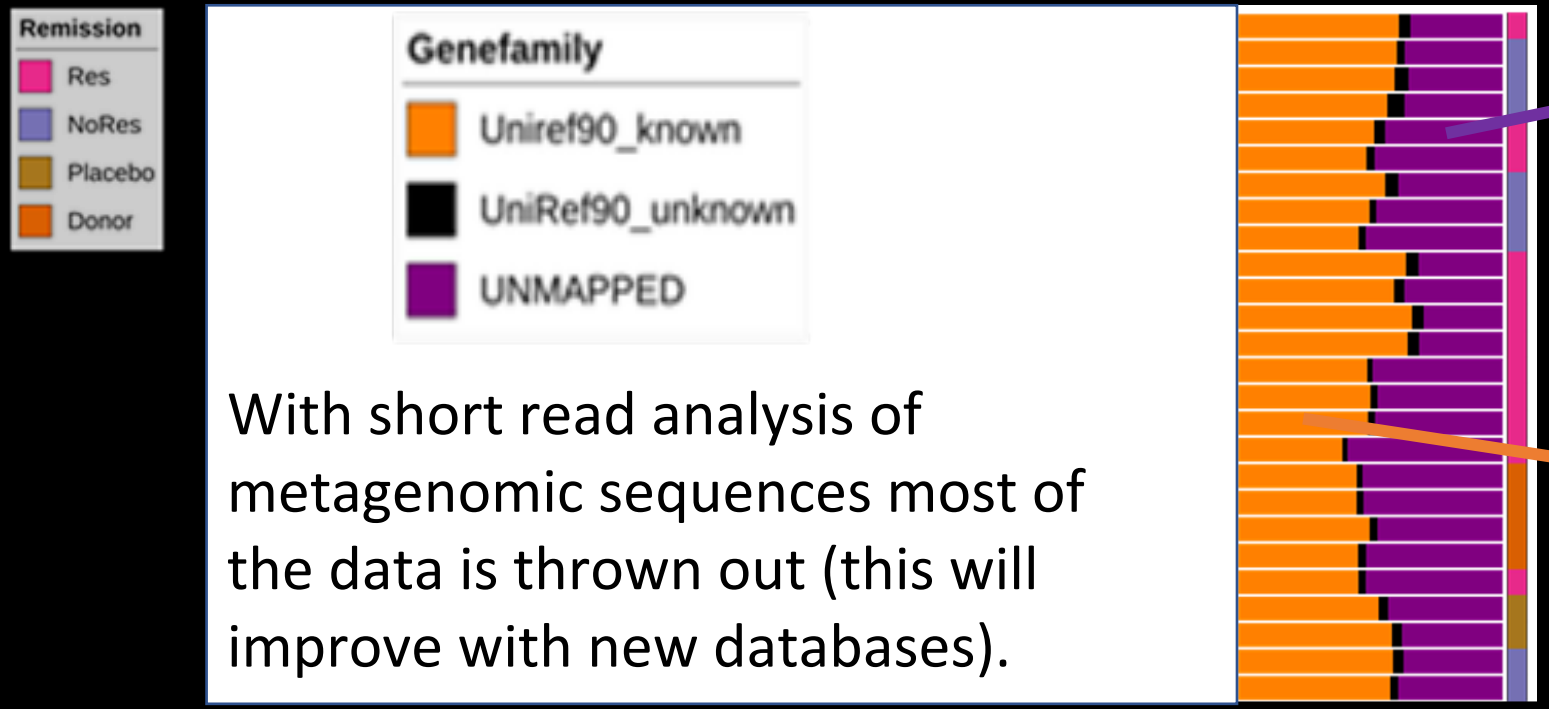


With short read analysis of metagenomic sequences most of the data is thrown out (this will improve with new databases).

Basically this data is thrown out – can be over 50% o the data

Functionally assigned but often at a very descriptive level and limited functional detail.

Sharok Shekarriz, unpublished

Methodologies for Analysis the Microbiota

Shotgun Metagenomics

Improved databases of isolated strains (WGS) and assembled metagenomic data (MAGs) will greatly improve functional characterization of metagenomic data - using read mapping approaches.

A unified catalog of 204,938 reference genomes from the human gut microbiome
Almeida et al Nature Biotechnology (2020)

'More than 70% of the UHGG species lack cultured representatives, and 40% of the UHGP lack functional annotations'

Large-Scale Analyses of Human Microbiomes Reveal Thousands of Small, Novel Genes
Sbero H et al (2019) Cell

*" **Over 90% of the small protein families have no known domain and almost half are not represented in reference genomes.** "*

An Integrated Metagenome Catalog Reveals New Insights into the Murine Gut Microbiome 2020
Lesker et al Cell Reports 30(9):2909-2922.e6

An expanded gene catalog of the mouse gut metagenome. Zhu et al bioRxiv preprint (2020)

" ... and 30% were functionally annotated."
That means 70% of genes have no known function!

Culture and Culture-Enriched Metagenomics

The human microbiome is mostly readily cultured

The diversity of the human gut microbiome recovered by culture is greater than either 16S profiling or metagenomics

Comprehensive culturing approaches under diverse conditions provides a framework for target culturing specific strains/species

Lau et al 2016 Gen Medicine.

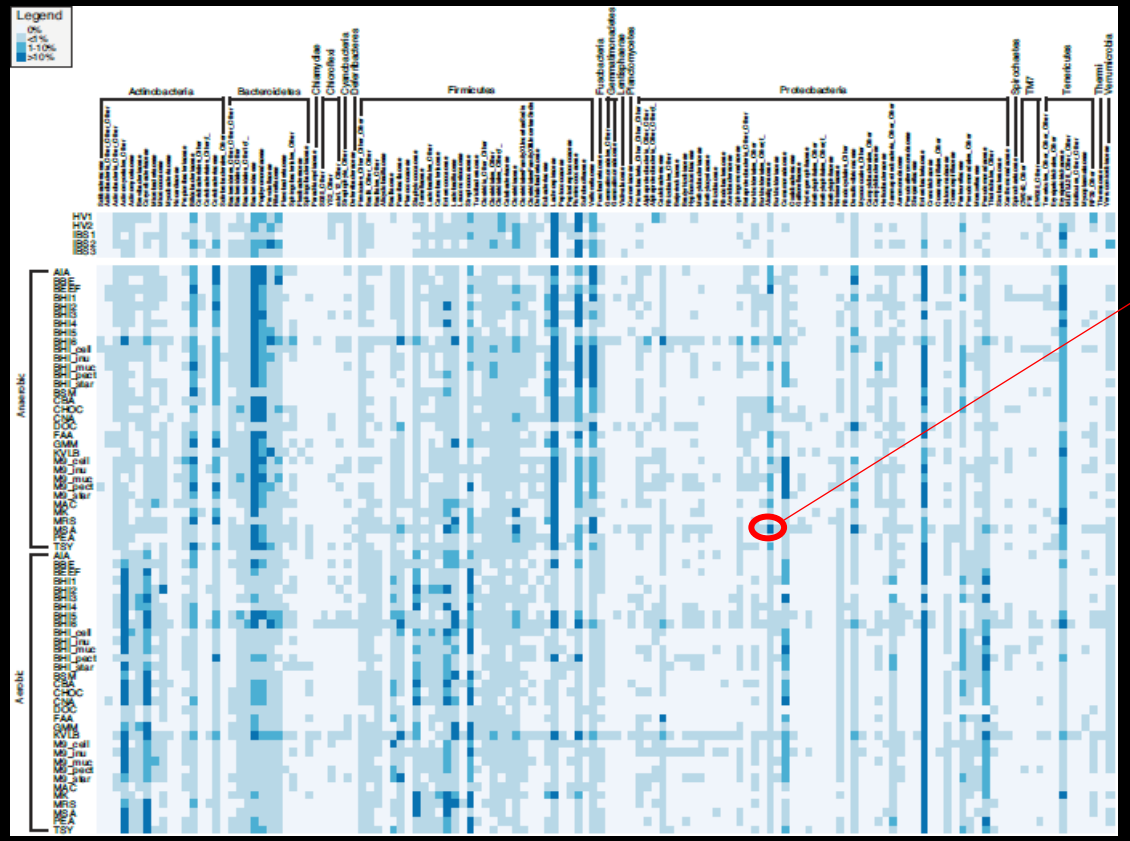
Methodologies for Analysis the Microbiota

Culture and Culture-Enriched Profiling (16S)

culture conditions x bacterial taxa

Bacterial Family

Growth Conditions



Targeted Culturing

Best condition for this bacterial Family /Genus / Species

Under continuous refinement…

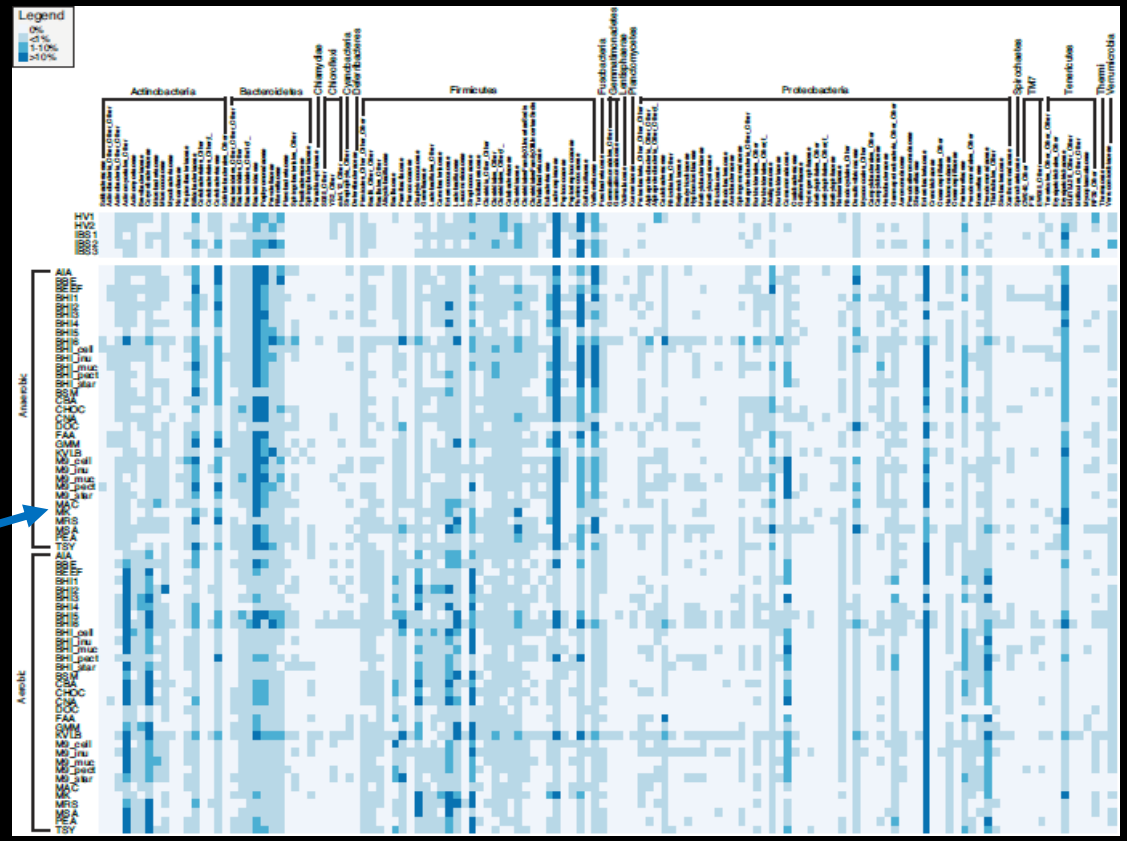
Culture and Culture-Enriched Metagenomics

Adding metagenomics to each culture conditions means we can look for specific strains or even specific genes

Bacterial Family

Cholesterol Metabolism by Uncultured Human Gut Bacteria Influences Host Cholesterol Level
Kenny et al 2020 Cell Host & Microbe

These genes are specific to and highly enriched on this culture condition



Under continuous refinement…

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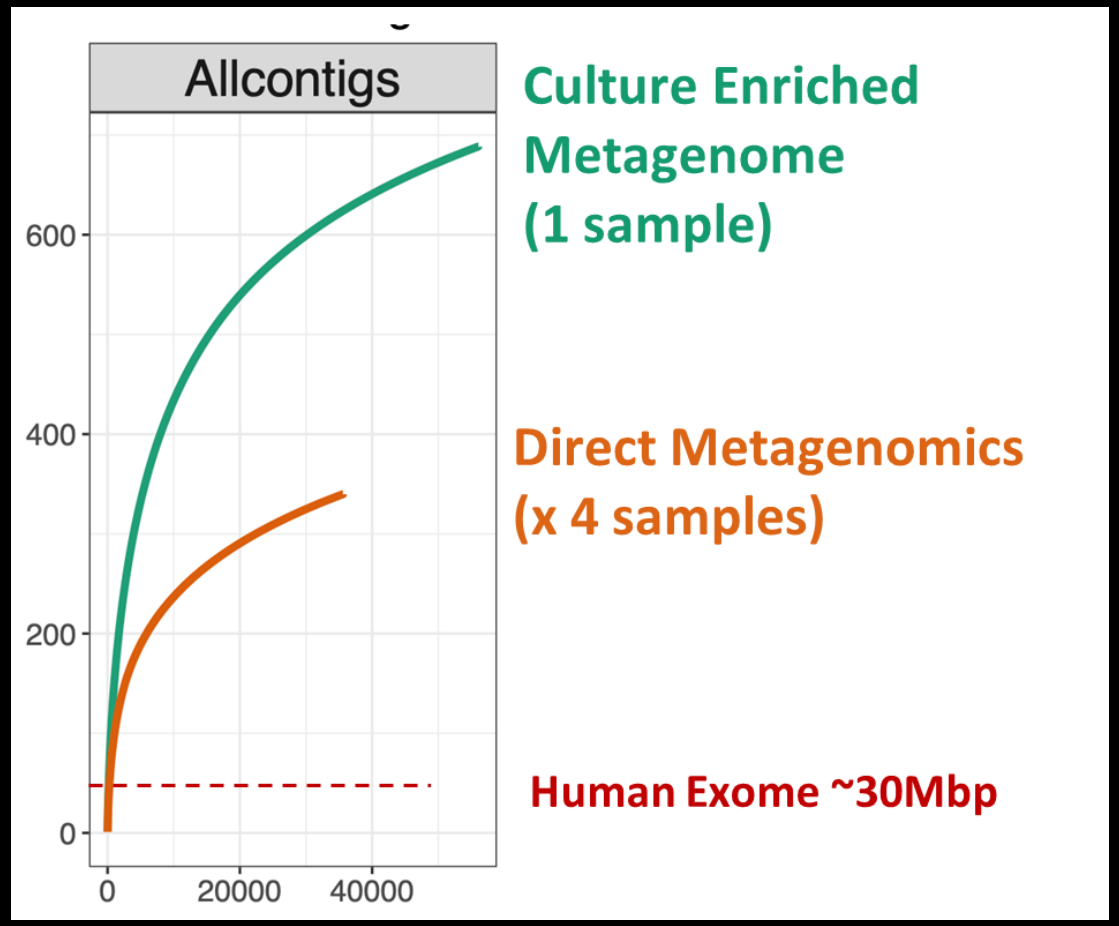
Culture and Culture-Enriched Metagenomics

Assembled Contigs > 2.5kbp (Ave gene 1kb)



206 MAGs

272 BINs

45 MAGs
120 BINs

Metagenomic Assemble Genomes (Strain level resolution)

incomplete or mixed metagenomic assemble genomes

Donor B

Using this database we have found 3 strains that account for over half of the engrafted genes in FMT Responders in our UC FMT study

Sharok Shekarriz, unpublished

Methodologies for Analysis the Microbiota

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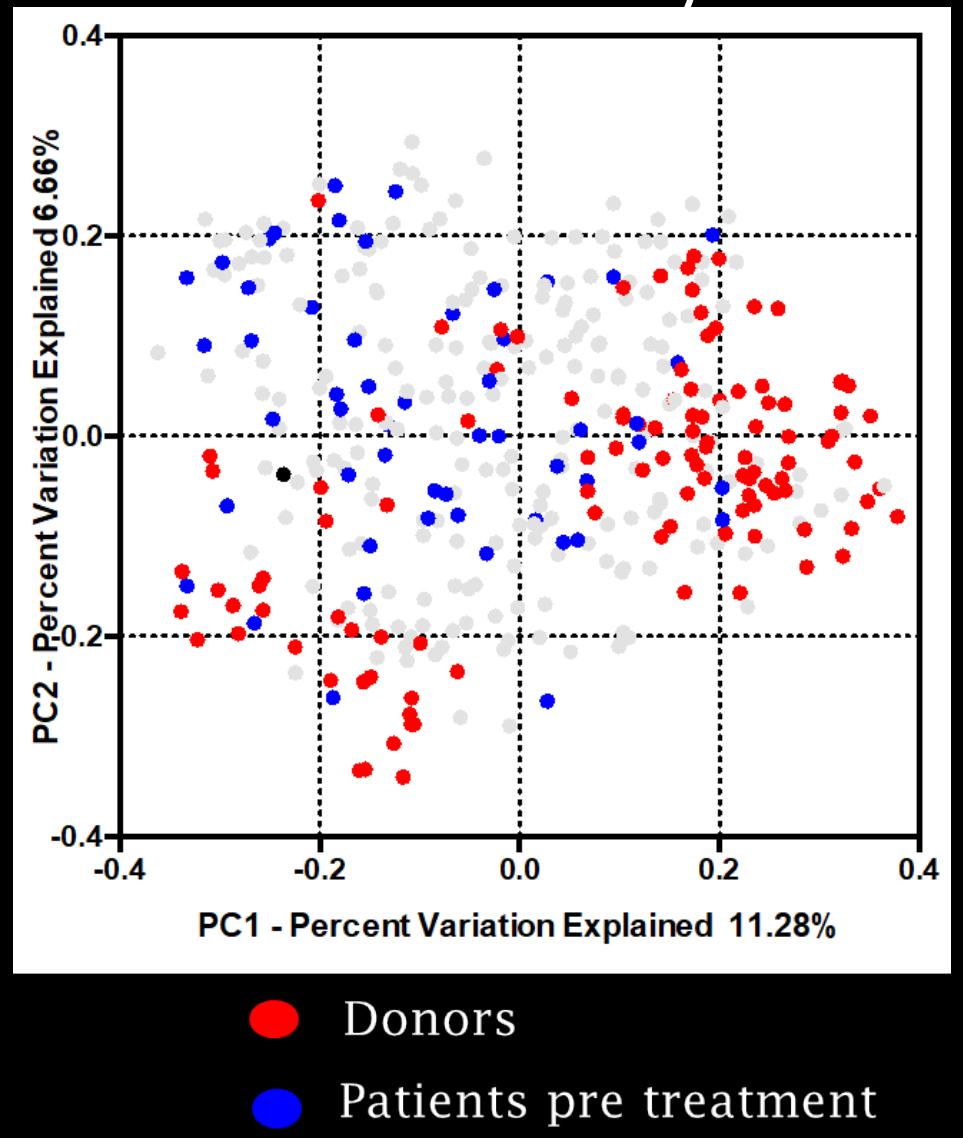
- All the approaches have advantages and limitations
- Analytical tools and databases (particularly for metagenomics) are rapidly changing
- The biggest datasets are not always the best for a particular question
- Exploring these rich datasets requires more than running through standard pipelines and finding what's different between two groups

Extra Slides

Every individual has their own unique microbiome.

This intrinsic heterogeneity makes it difficult to distinguish healthy from "*dysbiotic*" microbiomes

UC Patients vs Healthy Contols



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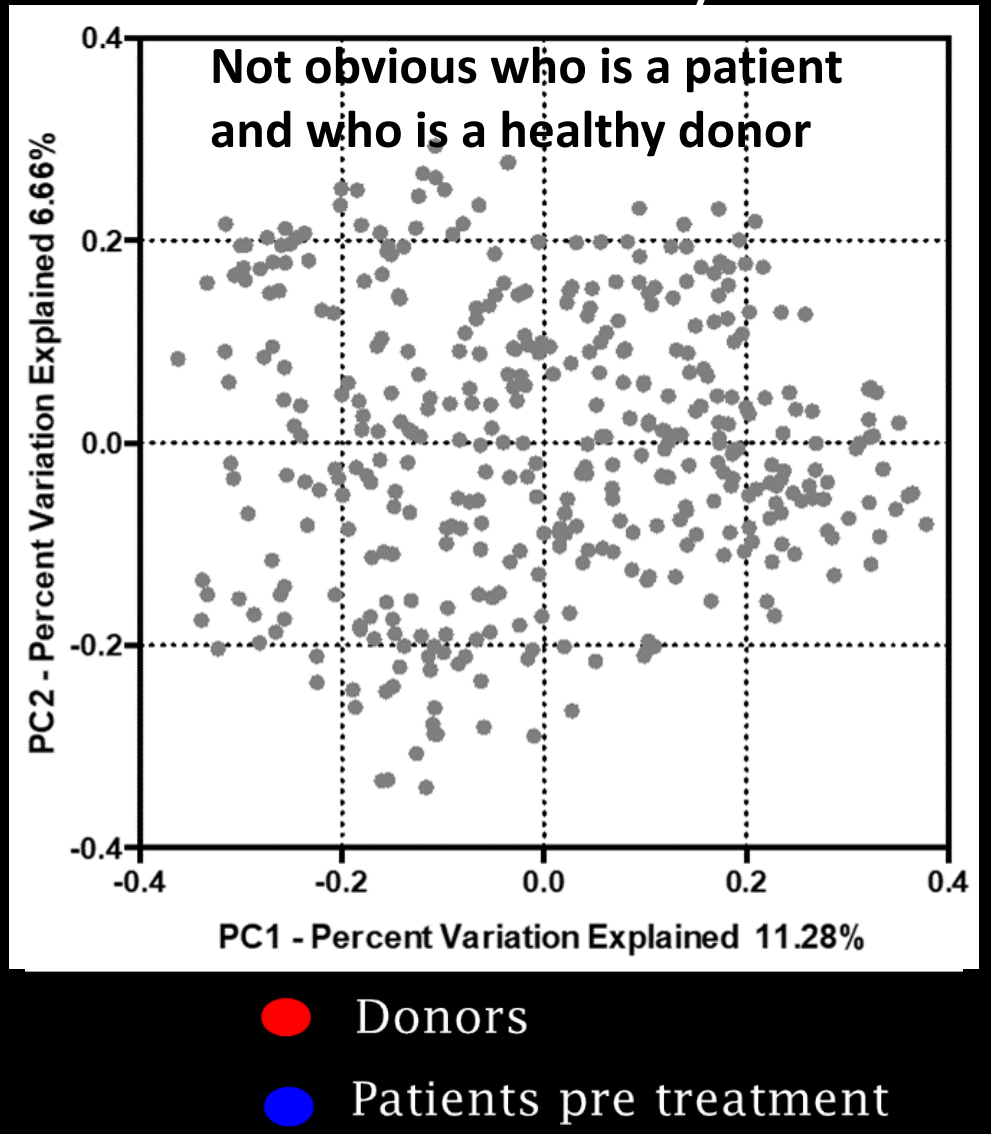
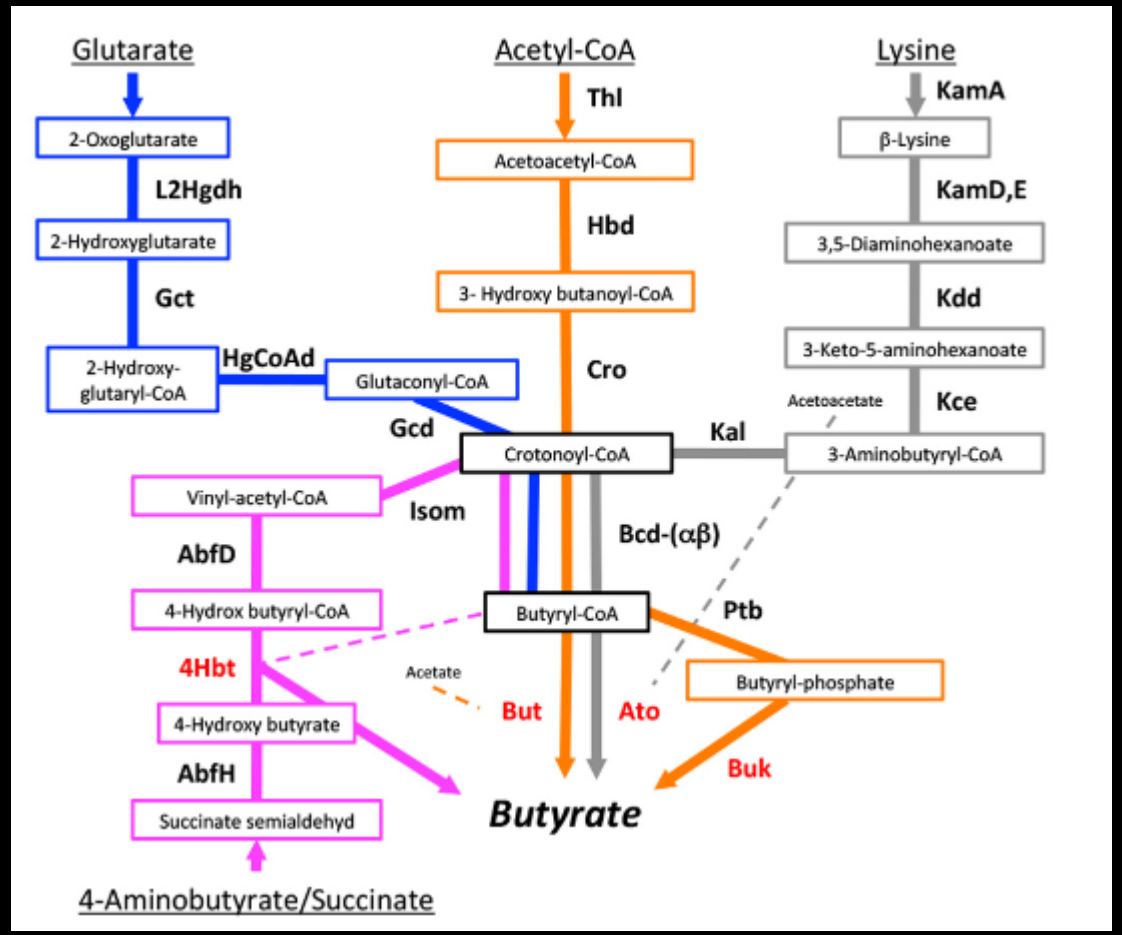


Redundancy

4 bacterial pathways for butyrate synthesis



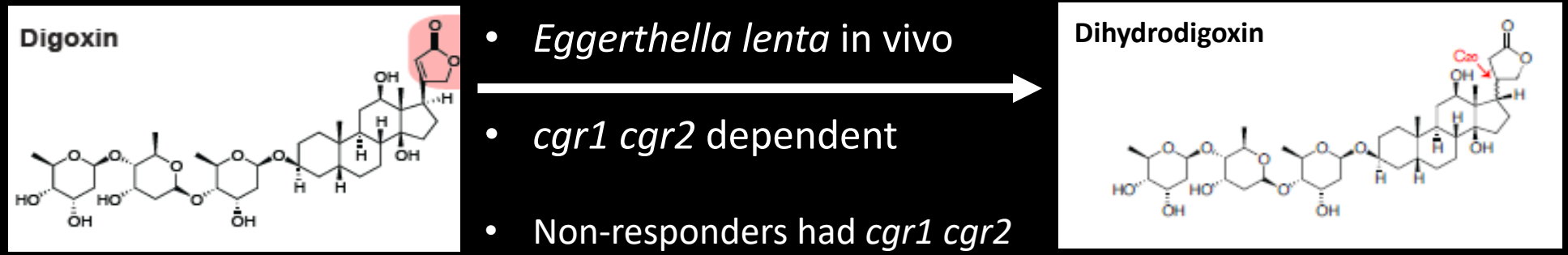
There are many Genera (each represented by multiple species) that are known butyrate producers

Faecalibacterium
Anaerostipes
Eubacterium
Roseburia
Coprococcus
Subdoligranulum
…

Vital M et al. (2014) mBio 5(2):e00889-14.

Specificity

- Digoxin is a medication used to treat various heart conditions
- Known that there are responders and non-responders
- Some patients excrete the inactive digoxin metabolite dihydrodigoxin
- Co-administration of broad spectrum antibiotics increases serum digoxin
- *Eggerthella lenta* reduces digoxin in vitro



Only 1 of 3 isolates of *E. lenta* had *cgr1 cgr2*
and were capable of inactivating digoxin

ie. Strain not Species dependent

Haiser HJ et al , *Science*. 2013