

The study of gut microbiota using the 16S rRNA gene

Shinichi Sunagawa / Guillem Salazar

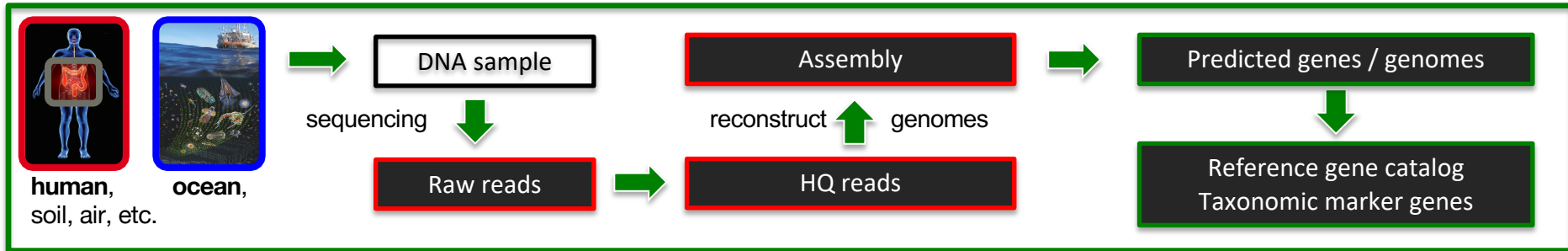
Overview

1. Studying microbiota by 16S rRNA gene analysis
2. Human gut microbiome in health and disease
3. Mice as model organisms for the study of the microbiota

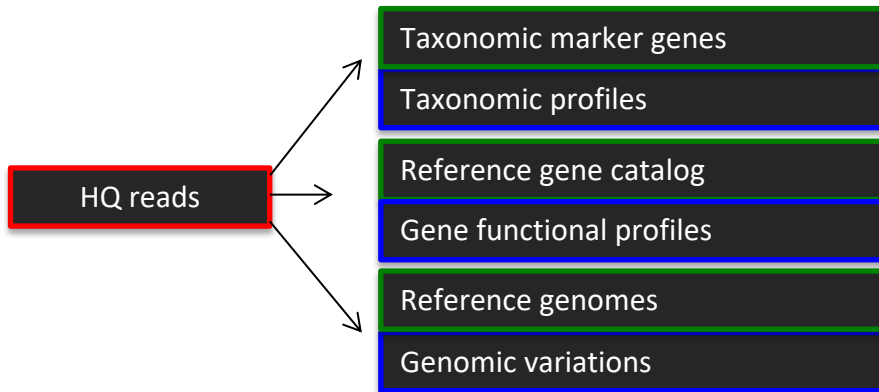
Terminology

- **Individual** – organism of specific genotype (e.g., bacterial strain)
- **Microbial ‘species’** – a group of co-existing strains that display greater genotypic similarity within themselves than to strains in other groups; often operationally defined based on sequence similarity of taxonomic marker genes (e.g. 97% 16S rRNA gene): → **OTU - operational taxonomic unit**
- **Microbiota** – microbial taxa that populate a given habitat (e.g. gut microbiota)
→ Taxonomic marker genes
- **Microbiome** – microbial taxa and their genomic content (e.g. gut microbiome)
→ Metagenomics

Metagenomics analysis



Quantitative analyses



'Who is there?'

- species composition
- community structure

'What do they do?'

- functional potential

'How do variable are individuals?'

- gene content differences

'How are populations structured?'

- biogeography of individual species

Kultima, et al., PLoS One 2012 & Bioinformatics 2016; Sunagawa, et al., Nature Meth, 2013;

Mende, Sunagawa, et al., Nature Meth, 2013; Schloissnig, et al., Nature, 2013; Nielsen, et al., NBT, 2014; Li, et al., NBT, 2014

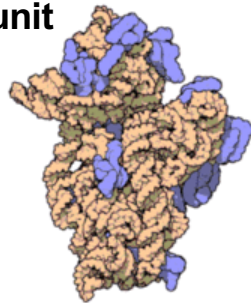
The 16S rRNA gene

- Part of prokaryotic ribosomes

50S large subunit (33 proteins)	<u>5S</u> : 120 nt <u>23S</u> : 2906 nt
30S small subunit (22 proteins)	<u>16S</u> : 1542 nt

- 16S rRNA present in all prokaryotes

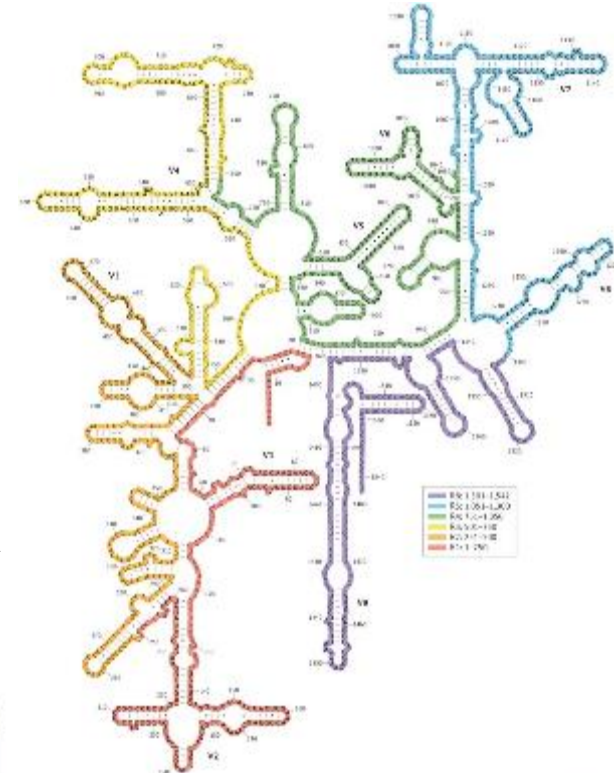
30S small subunit



blue: ribosomal proteins
gold: 16S rRNA



Secondary structure of 16S rRNA



- conserved regions and variable regions



CONSERVED REGIONS: unspecific applications

VARIABLE REGIONS: group or species-specific applications

Popular 16S rRNA gene databases



<https://rdp.cme.msu.edu/>

University of Michigan



<http://greengenes.lbl.gov>

LBLN Berkeley,
now second genomes



<https://www.arb-silva.de/>

Technical University Munich,
now MPI Bremen

Taxonomy: Kingdom, Phylum, Class, Order, Family, Genus, Species, (Strain)
But...NCBI, Bergey, SILVA, Greengenes, ...

Popular 16S rRNA gene analysis tools



Mothur
<https://www.mothur.org/>

University of Michigan



USERCH
<http://www.drive5.com/usearch/>

Robert Edgar



QIIME
<http://qiime.org/>

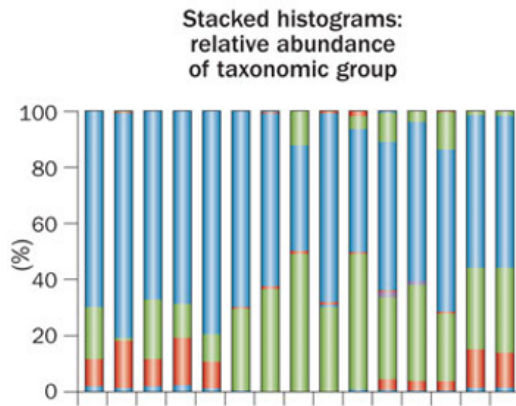
University of Colorado



DADA2
<https://benjjneb.github.io/dada2/>

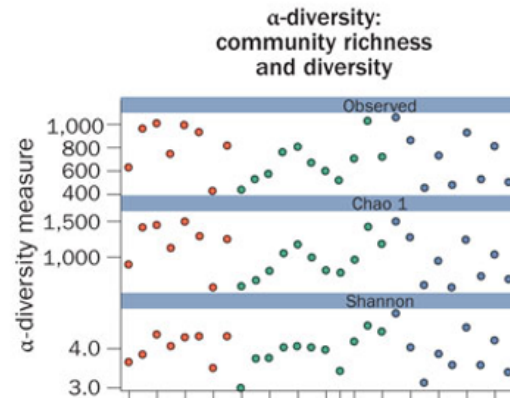
Benjamin Callahan

Community structure analysis



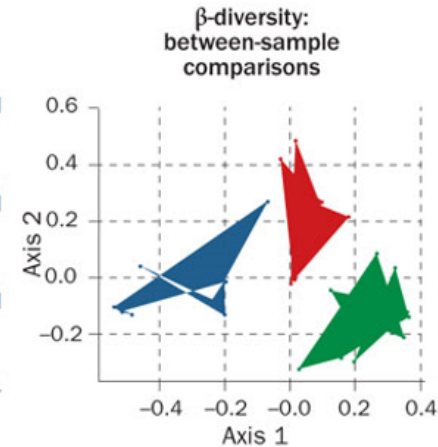
Relative abundances

note: suffers from
problem of compositionality



Alpha-diversity

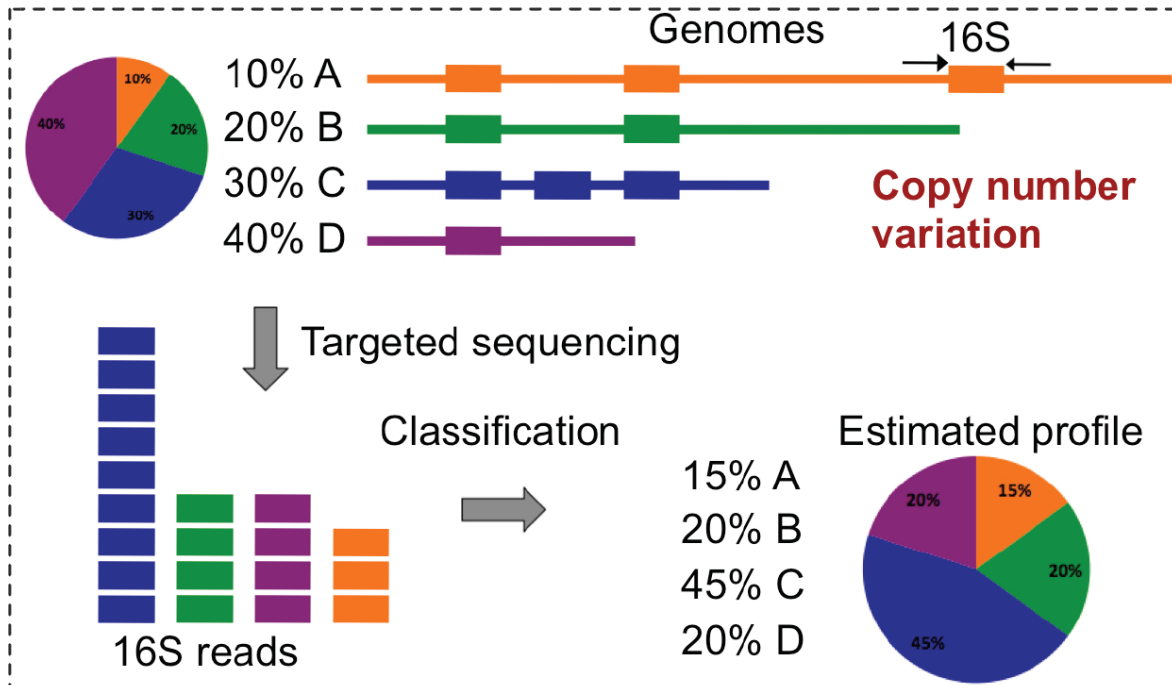
ecologically defined as
a function of the number of
species (richness) and the
evenness of species distribution



Beta-diversity

analysis of between-
community dissimilarity

Caveat of multiple 16S rRNA gene copies



(a) Targeted sequencing of 16S rRNA

See also: <https://rrndb.umms.med.umich.edu/>

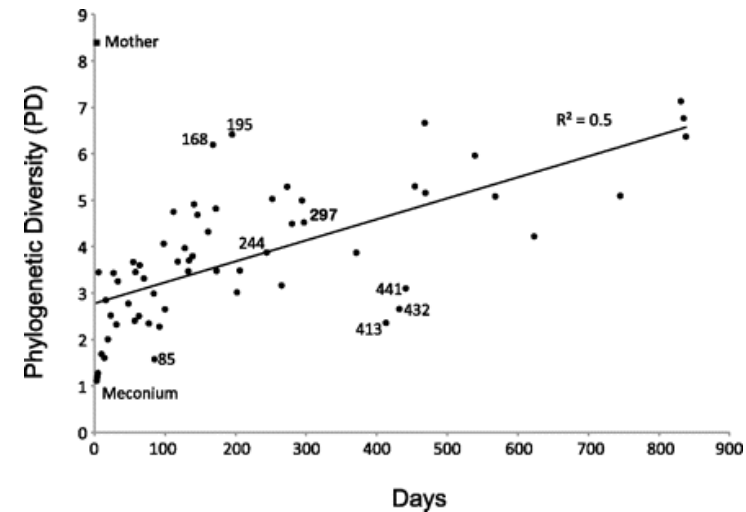
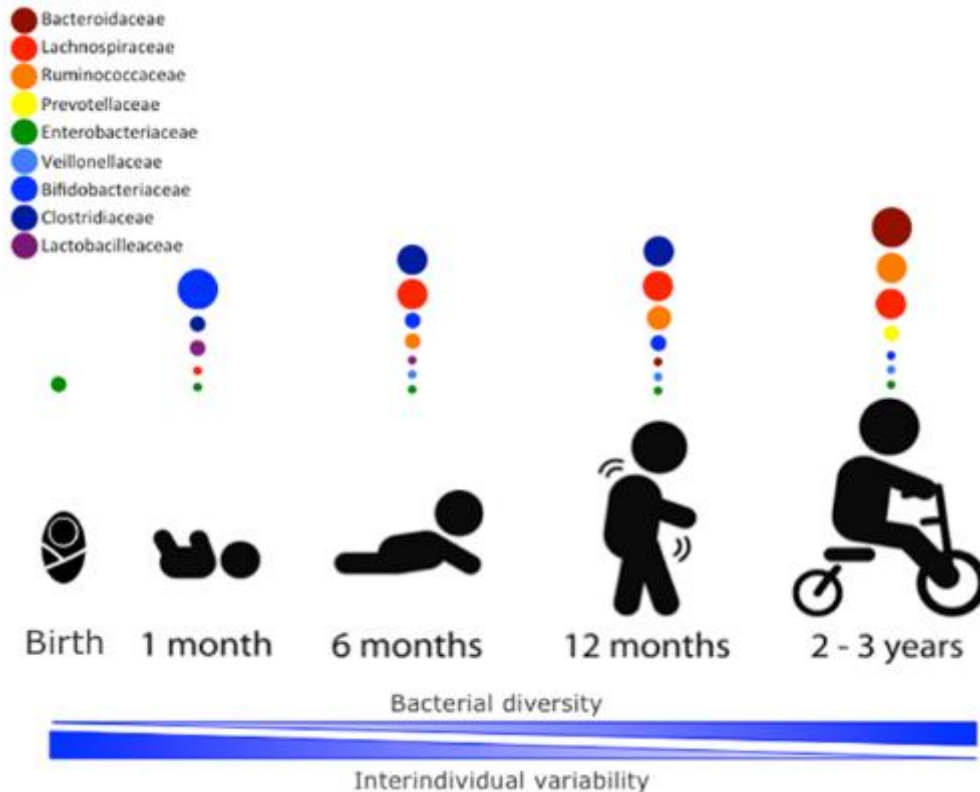
The human microbiome



- Antonie van Leeuwenhoek (microscope → microbiology) studied differences between oral and fecal ‘animalcules’ in 1680s
- Term “microbiome” coined in 2001 by Joshua Lederberg
 - What is it to be human?
 - What is the microbiome?
- Largest population resides in gastrointestinal tract: the gut microbiome
 - “How many gut microbial species exist?” (problems with definitions of bacterial species)
 - “How does the gut microbiome vary over time and between individuals?”
 - “How does the gut microbiome of different sites in the same person compare to the those in other people?”

Development of the gut human microbiome

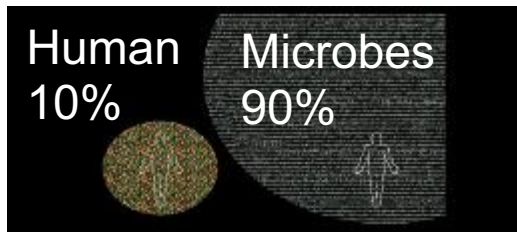
- Role of birth mode, diet change, antibiotics treatment



- ➔ alpha-diversity increases
- ➔ beta-diversity decreases

Human gut microbiome in numbers and time

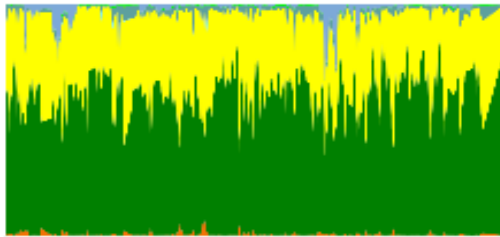
- Outnumber human genetic variation



now revised to about 1:1
(Sender et al. 2016)

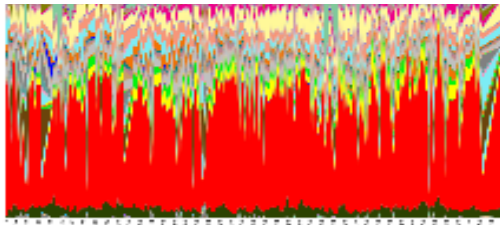
Vast majority in gut

- 100's (metagenomics) to 1000's (16S) of OTUs
- 3 - 10 mio genes (while only ~300k prevalent)
- 10 mio SNVs in 100 species in 200 individuals



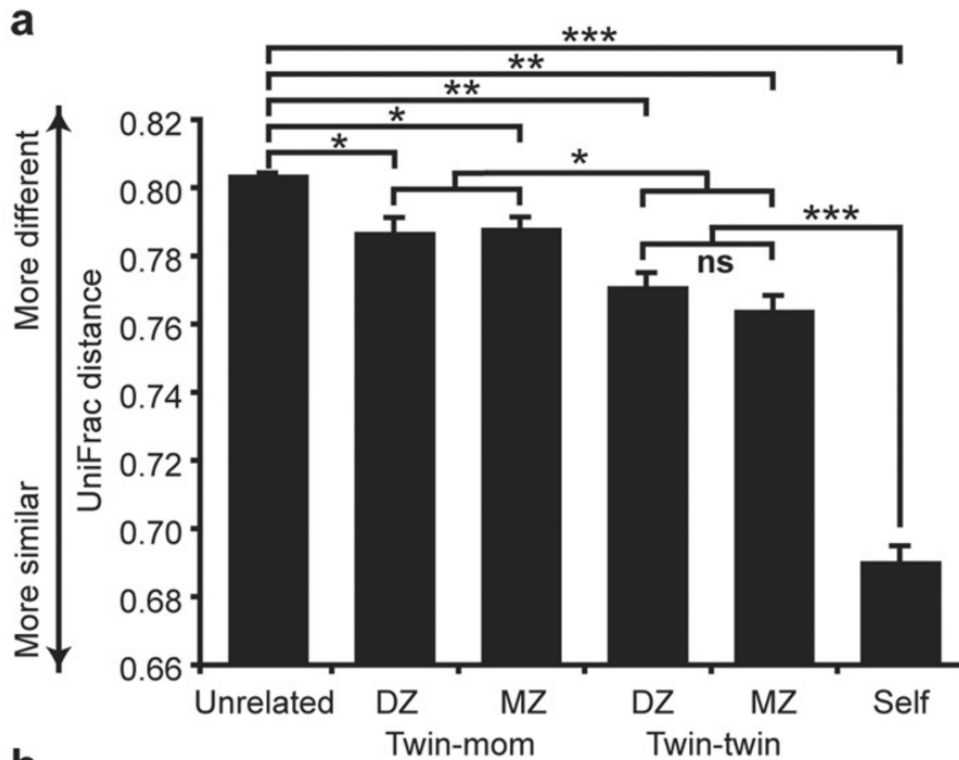
Temporal variability depends on taxonomic resolution
>1yr daily stool sample sequencing

→ Phylum-level



→ OTU-level

Compositional variability between individuals



Human gut microbiome in health and disease

Gut Microbiota from Twins Discordant for Obesity
Modulate Metabolism in Mice

Ridaura et al, Science, 2013

Obesity

Disentangling the effects of type 2 diabetes
and metformin on the human gut microbiota

Forslund, et al., Nature, 2016

Diabetes

**Richness of human gut microbiome
correlates with metabolic markers**

Le Chatelier, et al, Nature, 2013

Metabolic
syndrome

**Microbiota Modulate Behavioral and
Physiological Abnormalities Associated
with Neurodevelopmental Disorders**

Hsiao et al, Cell, 2013

Autism

And other diseases

Crohn's, Ulcerative
Colitis, MS, Parkinson,

→ Colorectal cancer

**Alterations of the human gut microbiome
in liver cirrhosis**

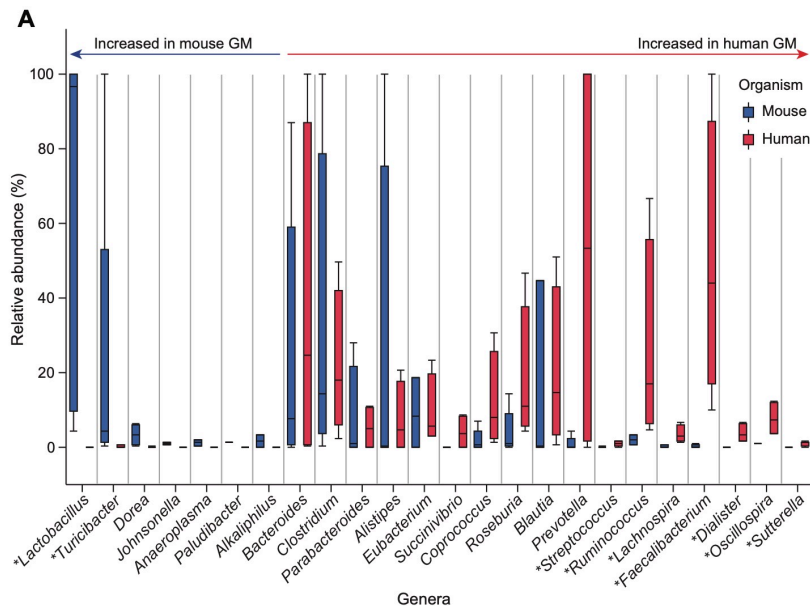
Qin et al, Nature, 2014

Liver cirrhosis

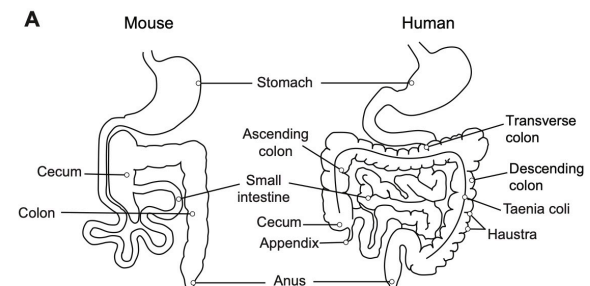
For obesity, causality (partly) established, for other diseases: correlation != causation

Mice as experimental model organisms in preclinical research

- They are small (housing),
- They are fast to breed (reproduction time of 3 weeks)
- They have an innate and adaptive immune system that is similar to the human immune system.
- They have similar anatomy (bigger Cecum)



Nguyen et al., 2015



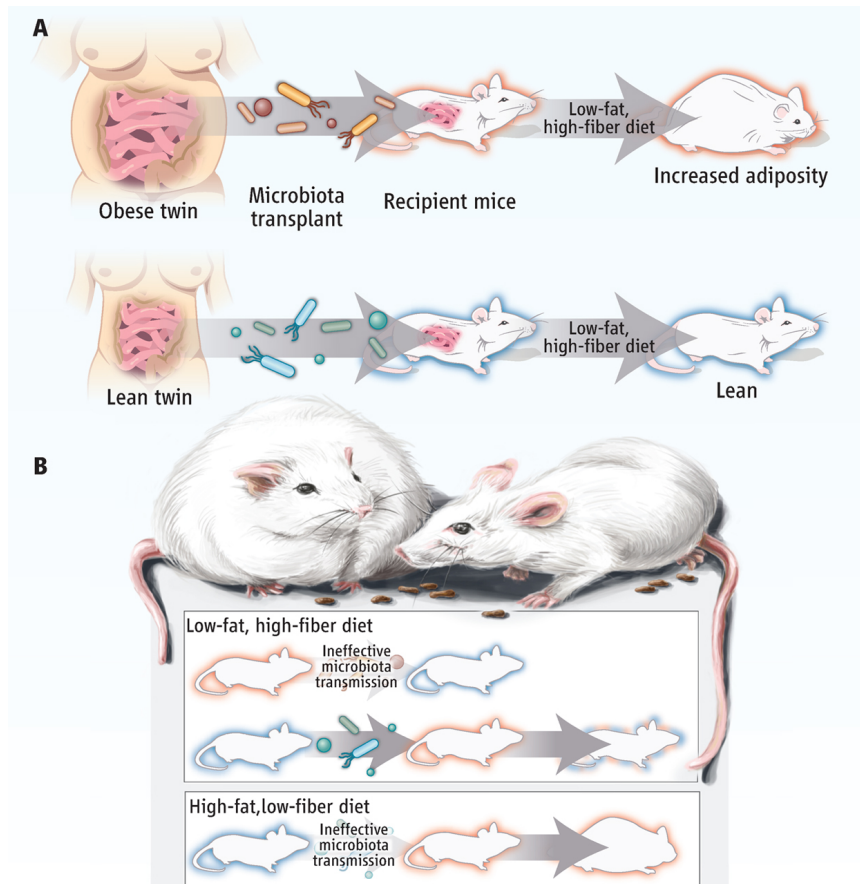
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 - They have similar anatomy (bigger Cecum)
 - It is relatively easy to produce genetically-engineered strains.
 - It is relatively easy to manipulate their microbiota.

 - **Germ-free mice** – mice that have no microorganisms living in/on them

 - **Gnotobiotic mice** – mice colonized with defined bacterial strains or mixtures
- instrumental to test causal relationships between microbes and their host

Microbiota and disease – obesity



Ridaura et al., Science, 2013
 Perspective by Walker and Parkhill 2013