Technologien zur Erforschung des Mikrobioms
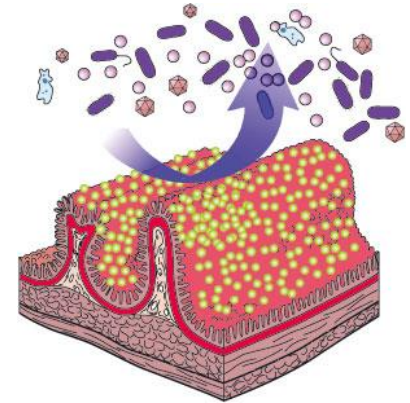
(Universität Kiel, Institut für Klinische Molekularbiologie)
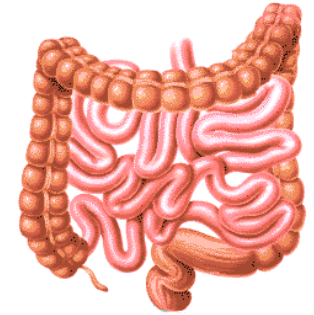
Ateequr Rehman

Microbiota

▶ Also termed normal flora and microflora

▶ Organisms (**bacteria**, virus, archaea and fungi) that colonize the body's surfaces:

- Skin
- Oral cavity
- Nasal cavity
- Vagina
- Gastro-intestinal tract

Compostion and structute is habitat specific
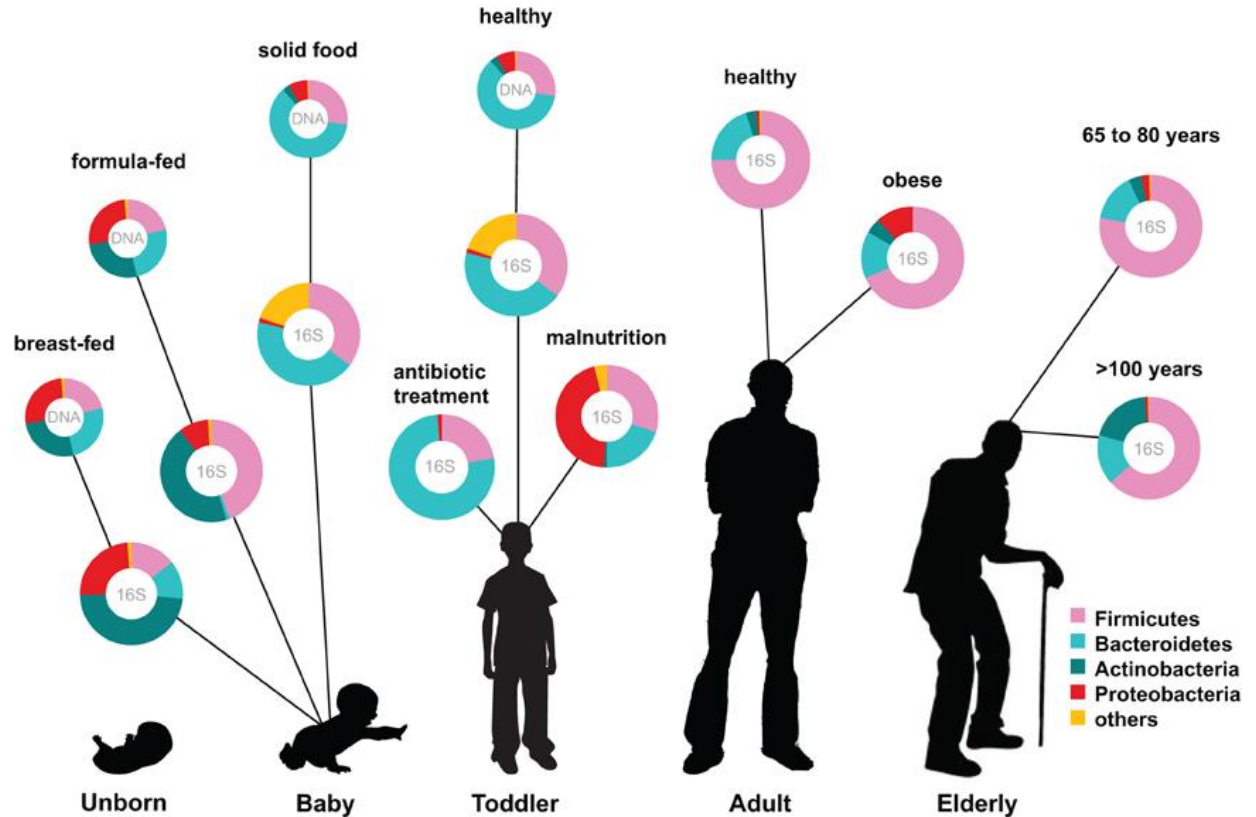
Complexity of microbiota

- ~100 billion (1×10^{11}) bacteria/gm feces

- ~ 500 to1000 bacterial species

- ~ 4000 genes per genome

- ~ 2 million to 4 million genes

- ~ 50 to 100 times as many as our "own" genome.

Composition of microbiota

▶ Mostly strict anaerobes

Key phyla:

- Bacteroidetes
- Firmicutes,
- Proteobacteria
- Verrucomicrobia
- Actinobacteria
- Fusobacteria
- Cyanobacteria

Noora Ottmann, Front. Cell. Infect. Microbiol., 09 August 2012

What controls the microbiota

Mode of delivery	Babies born by Caesarean section with delayed, reduced or absent colonization with *Bifidobacterium*, *Lactobacillus* and *Bacteroides*, and higher numbers of the *Clostridium difficile* group I, compared to vaginally born babies
Infant feeding	Exclusively formula-fed infants are colonized more frequently with *Escherichia coli*, *C. difficile*, *Bacteroides* spp. and lactobacilli than breast-fed children
	Breast-fed infants with higher cell counts and diversity in the *Bifidobacterium* microbiota
Ageing	Increase in *Enterobacteriaceae* and *Bacteroidetes*, reduced levels of *Bifidobacterium* spp.
Antibiotics	Antibiotic treatment results in rapid loss of diversity and a pronounced community shift, recovery after treatment is incomplete even after months
Diet	High-fat diet is associated with an increase in faecal *Enterobacteriaceae* and LPS levels in serum, resulting in insulin and glucose resistance and obesity
Type 2 diabetes	Reduced *Firmicutes* and clostridia compared to healthy controls
Obesity	Changes in the relative proportions of *Bacteroidetes* and *Firmicutes*
Inflammation	Increase in the abundance of bacteria belonging to the *Enterobacteriaceae* and reduction in *Faecalibacterium prausnitzii* in human patients and animal models of chronic and infectious intestinal inflammation
Host genotype	TLR5-deficient mice display changes in the levels of *Bacteroidetes* and *Firmicutes* and show hyperlipidaemia, hypertension, insulin resistance and increased adiposity
	MyD88 knockout mice show increased levels of *Lactobacillaceae*, *Rikenellaceae* and *Porphyromonadaceae* and are protected from type 1 diabetes
	Reduced diversity of the microbiota in patients suffering from familial Mediterranean fever

Importance

▶ Metabolic and Nutritional function

▶ Regulation of intestinal structure

▶ Maturation and function of mucosal immune system

▶ Nutrient acquisition and energy regulation

▶ Protection against pathogens
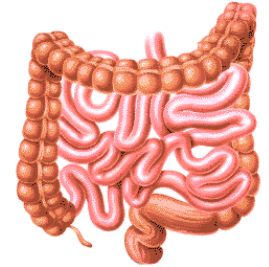
Microbiota study and experimental design

▶ phenotype representation
   does the disase manifest in the selected tissue?

▶ genotype, cage
   genetic background, non-genetic influences

▶ age
   influences various factors

▶ disease status
   onset, course…

▶ treatment
   antibiotics, DSS

▶ tissue location
   colon, ileum…

▶ diet
   influences metabolism etc.

▶ etc.

# Sampling: tissue and/or feces

Tissue /fecal sampling:

▶ Rinsing the tissue

▶ Number of washing or volume of buffer

▶ Cut the tissue in pieces of approx equal sizes

▶ Sample collection

▶ Storage solution

▶ Transportation

▶ and storge conditions

Sampling: Minimizing contamination

▶ Rinse tools in ethanol and water

▶ Use sterile set of of tool for each mice/organ
If sampling tissue from different location, or animal

▶ Start sampling from low to high microbial density
Skin ☐ oral cavity ☐ gut
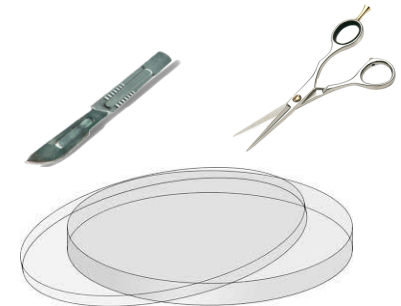
Microbiome analysis: The Tool Box

▶ Culture based methods: back bone

▶ Finger printing

▶ 16S rRNA gene cloning and sequencing

▶ Fluorescent in situ hybridization

▶ Real time quantification

▶ High throughput sequencing(16S rRNA and meta genomics/transcriptomics)
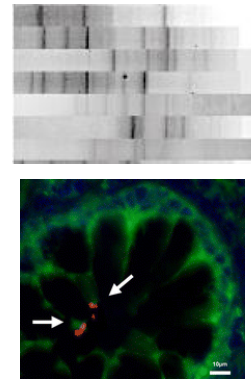
Microbiota: Extraction of nucleic acid

Feces and tissue

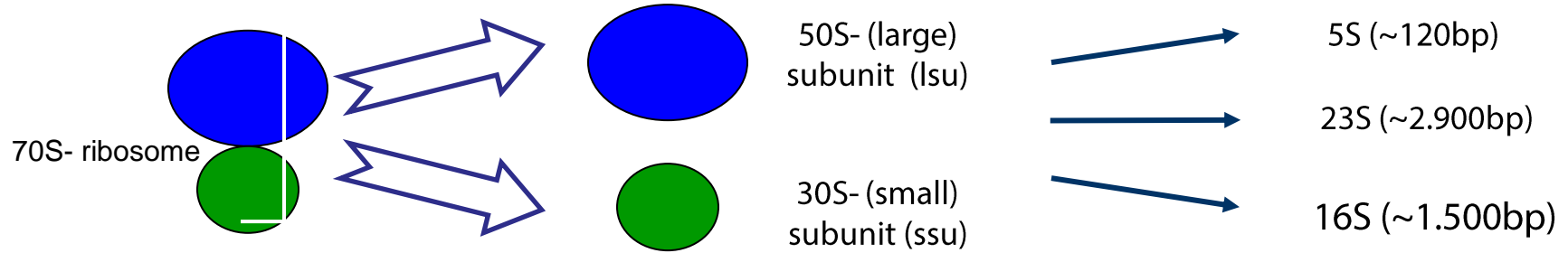Lysis: Chemical, physical and mechanical
Good PCR inhibitors technology

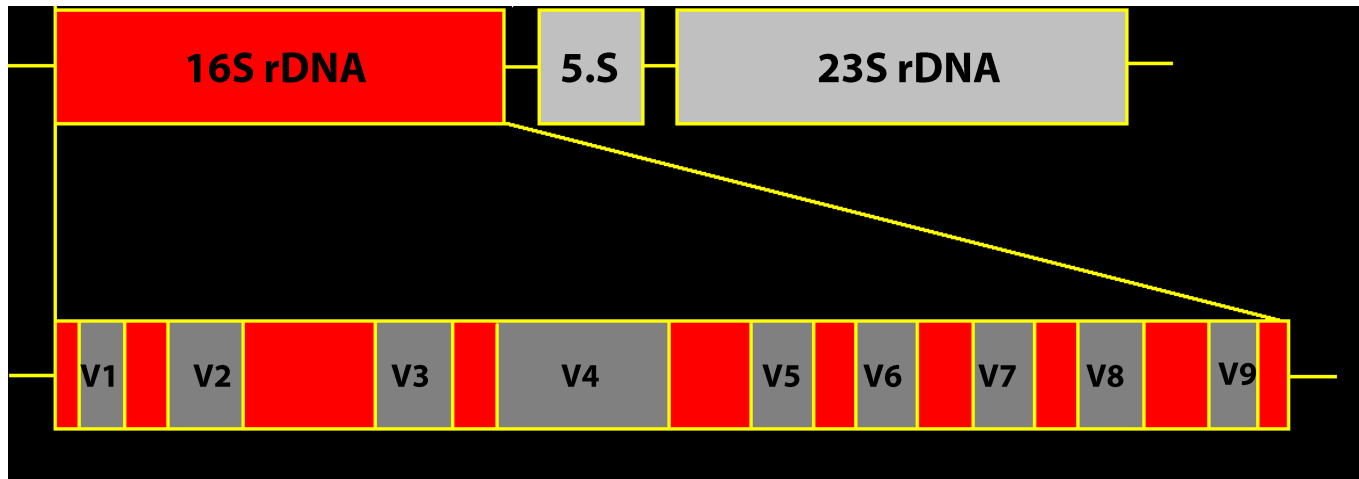DNA   RNA

standing   active microbial communities:

metagenomic   meta transcriptomics

water as negative control during extraction

Microbiota: 16S rRNA profiling

70S- ribosome

50S- (large) subunit (lsu) → 5S (~120bp)

23S (~2.900bp)

30S- (small) subunit (ssu) → 16S (~1.500bp)

Fingerprint „who is there ?": Sequence variability of variable regions

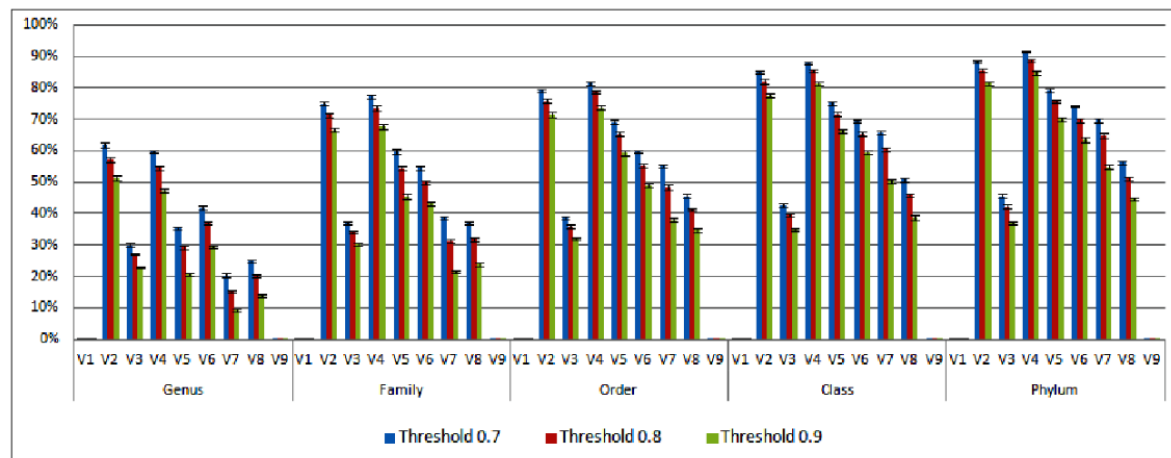| 16S rDNA	5.S	23S rDNA

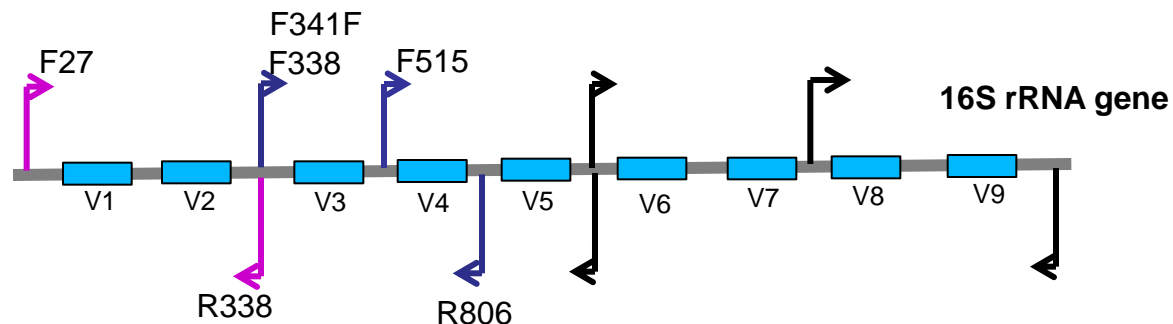| V1 | V2 | V3 | V4 | V5 | V6 | V7 | V8 | V9 |

Microbiota: 16S rRNA gene primers

▶ Taxonomic coverage

▶ Specificity

▶ Accuracy

Software for sequence analysis

▶ Contig formation
   (Mothur and QIIME)

▶ Quality control
   (Mothur and QIIME)

▶ Taxonomial classification: Phylum to species
   (Ribosomal data base project)

▶ Phylotype/OTU based analysis (Alpha, beta diversity)
      (Mothur , QIIME and RDP)

▶ Multivariate analysis (PcoA, CCA, PCA etc)
   Mothur , QIIME, Fast Unifrac, R package

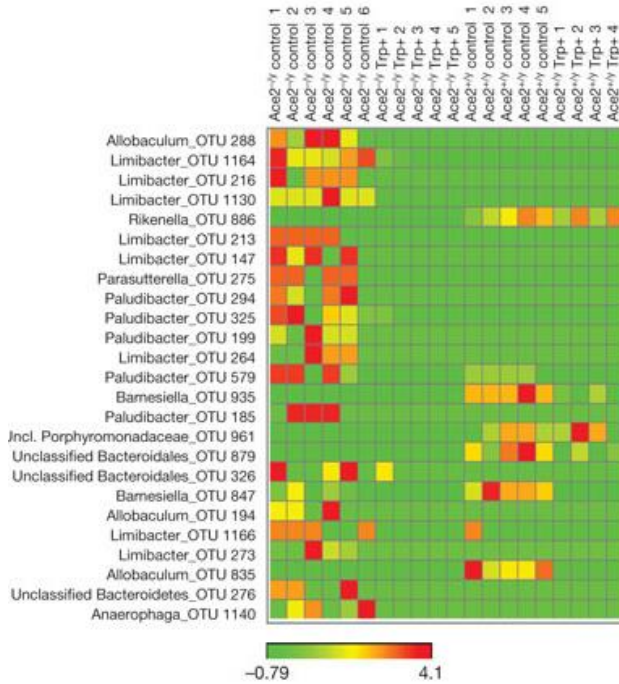# Microbiota data visualization:

Alpha diversity: within sample
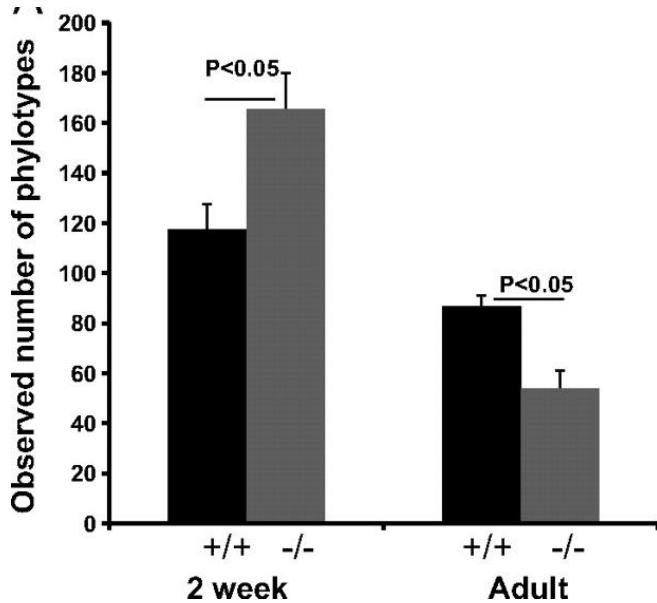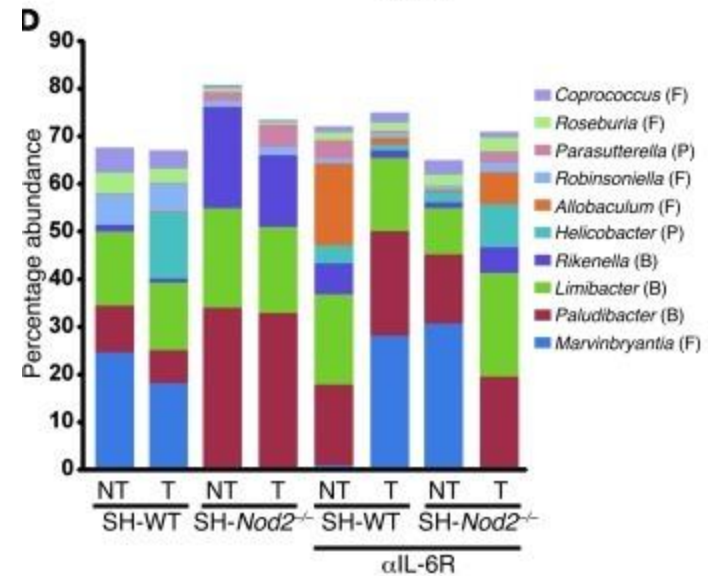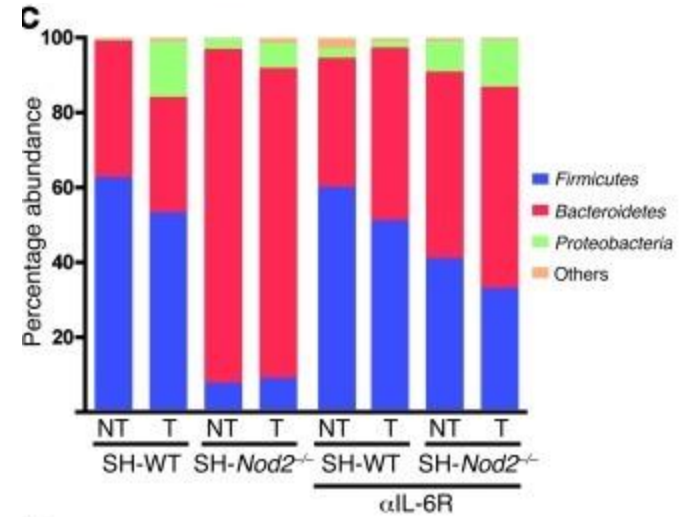
Beta diversity: between samples

Cluster and principle coordinate analysis
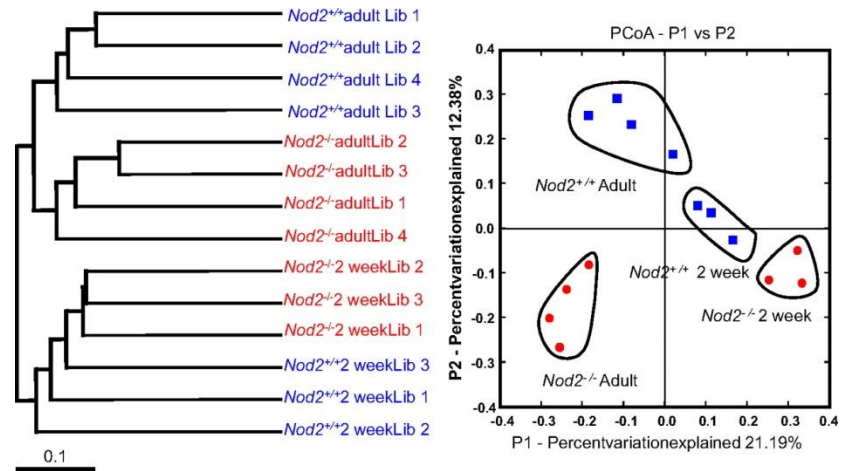
Microbiota data visualization:

Phylogenetic distribution

Heat map

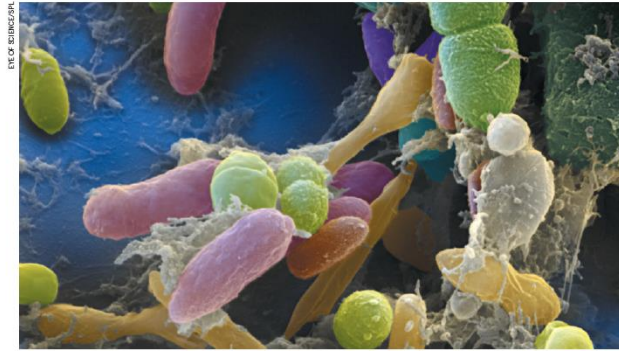**….. KILLER BACTERIAL COMMUNITY CAUSES CANCER**

….. THE BACTERIA BEHIND SMOG

…BACTERIA BEHIND HEALTH & LONGEVITY

…BACTERIA PREVENT STROKE

…..ANXIETY-BUSTING GUT BACTERIA

….BACTERIA MAKE US SMOKE

….. GUT BACTERIA KEEP YOU SLIM

A scanning electron micrograph of bacteria in human faeces, in which 50% of species originate from the gut.

Microbiome science needs a healthy dose of scepticism

To guard against hype, those interpreting research on the body's microscopic communities should ask five questions, says **William P. Hanage**.

Nature. 2014 Aug 21;512(7514):247-8

▶ Can experiment detect differences that matter ?

▶ Does the study show causation or just correlation ?

▶ What is the Mechanism ?

▶ How much do experiments reflect reality ?
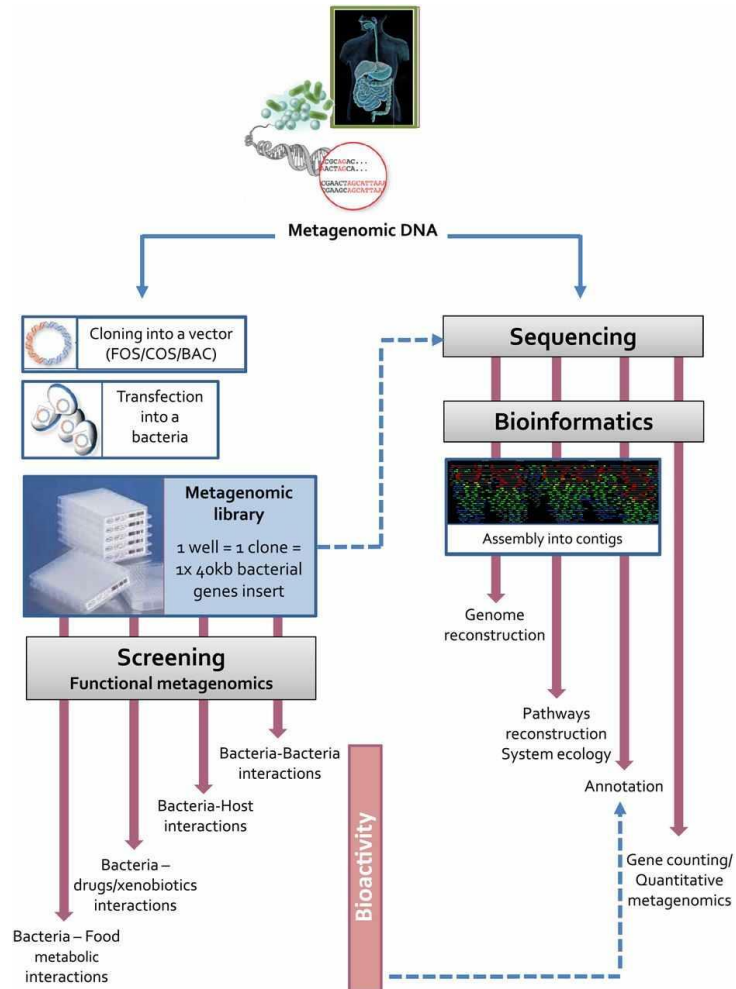
▶ Could anything else explain the results ?

Function ?

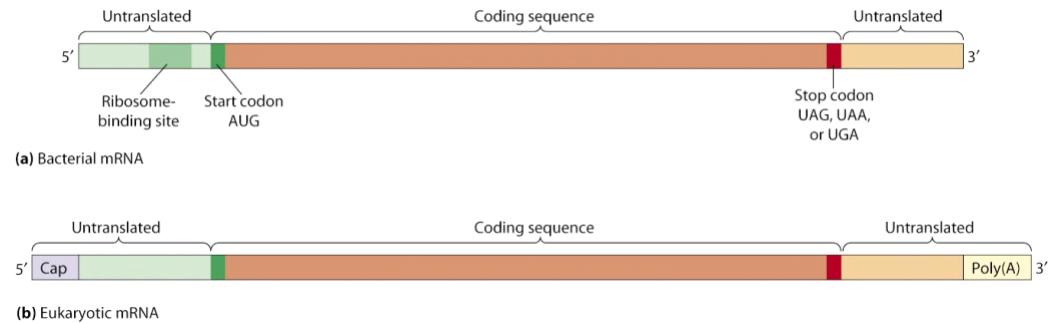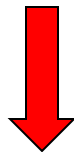# Complex: Metagenomics sensu strictu

Lepage et al., Gut 2013

Even more complex: Metatranscriptomics

bottle neck

▶ Prokaryotic mRNA lacks 3' end poly(A) tail

▶ Short half life prokaryotic mRNA

▶ rRNA and tRNA: ~95%

▶ mRNA: ~1-5%

(a) Bacterial mRNA

(b) Eukaryotic mRNA

Sequencing of Non enriched total RNA

Non mRNA sequences

Key points

▶ Microbiota is complex and placid component ? high hopes

▶ Protocol should be standardized to sample the material for microbiome analysis

▶ Several analytical tool but no consensus

▶ Only 16S rRNA gene profiling is not sufficient.

▶ Functional studies should be targeted in future

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