

Microbial communities and the impact of new generation sequencing (NGS) on paediatric infectious disease research

Learning outcome

You should be able to:

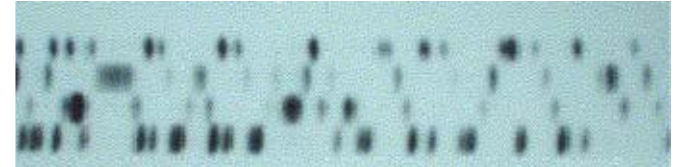
Give examples (and think of your own applications) of the use of high throughput sequencing technologies in paediatric infectious disease research

Sequencing methods

Late 1970s -

- Sanger

A
G
C
T



Late 2000s -

- 454, Illumina
- Pacific Bio, Ion Torrent...



The Archon X PRIZE for Genomics

US\$10 million prize:

"the first team that can build a device and use it to sequence **100 human genomes** within **10 days** or less, with an accuracy of no more than one error in every 100,000 bases sequenced, with sequences accurately covering at least 98% of the genome, and at a recurring cost of no more than **US\$10,000 per genome**."

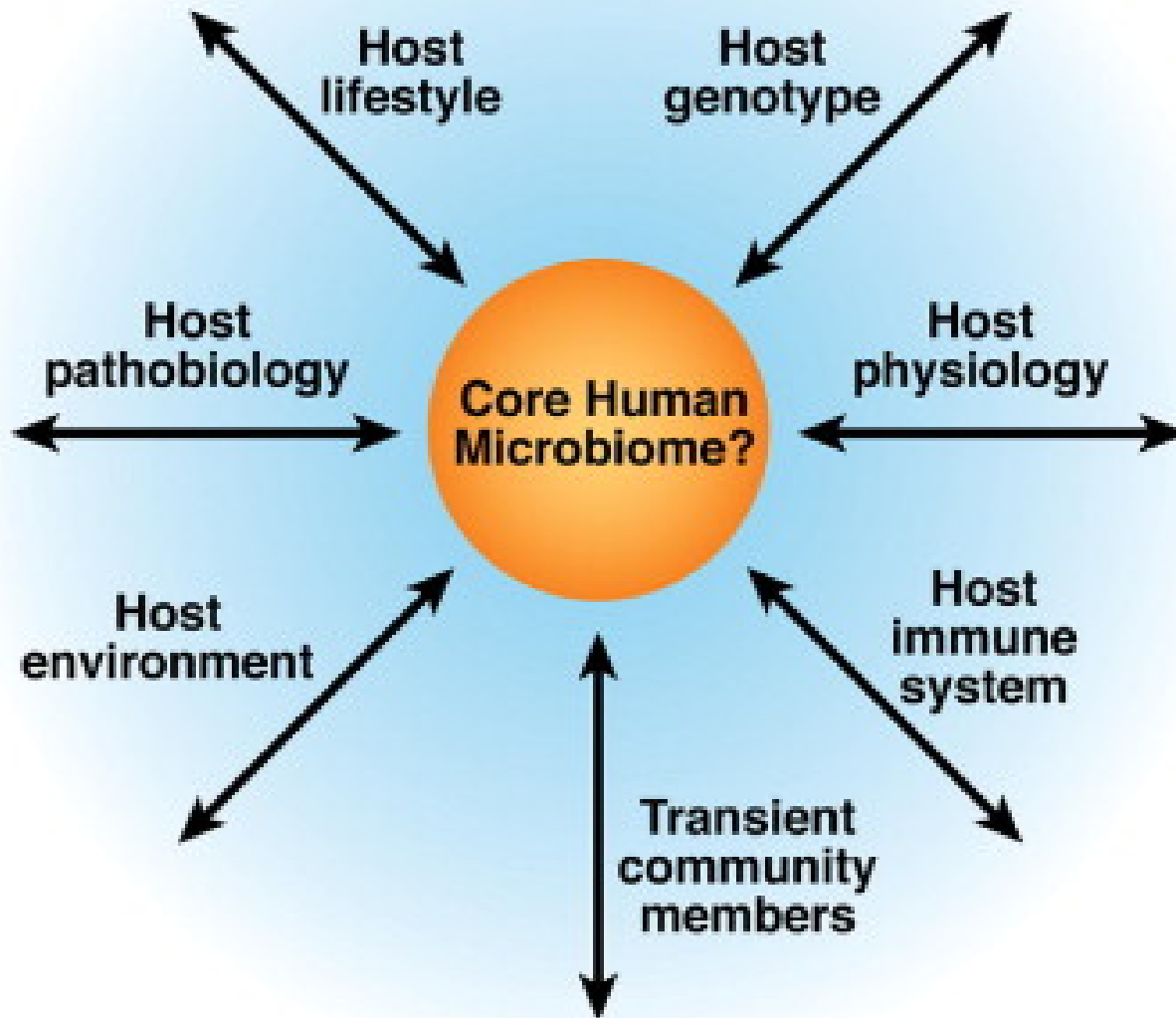
Method	Illumina	Pyrosequencing (454)	Ion semiconductor (Ion Torrent sequencing)	Single-molecule real-time sequencing (Pacific Bio)	Chain termination (Sanger sequencing)
Read length	50 to 250 bp	800 bp	200 bp	up to 2900 bp	400 to 900 bp
Accuracy	98%	99.9%	98%	87%	99.9%
Reads per run	600Gb	0.8Gb	60Gb	0.2Gb	N/A
Time per run	1 to 10 days	24 h	2 h	2 h	20 min-3 h
Cost per 1 million bases (in US\$)	\$0.05 to \$0.15	\$10	\$1	\$2	\$2400
Advantages	High sequencing yield	Large read size Fast	Cheap machine! Fast	Long read length	Long individual reads Accurate
Disadvantage	Short read length	Runs are expensive Homopolymers	Homopolymers	High error rate	Cost Time

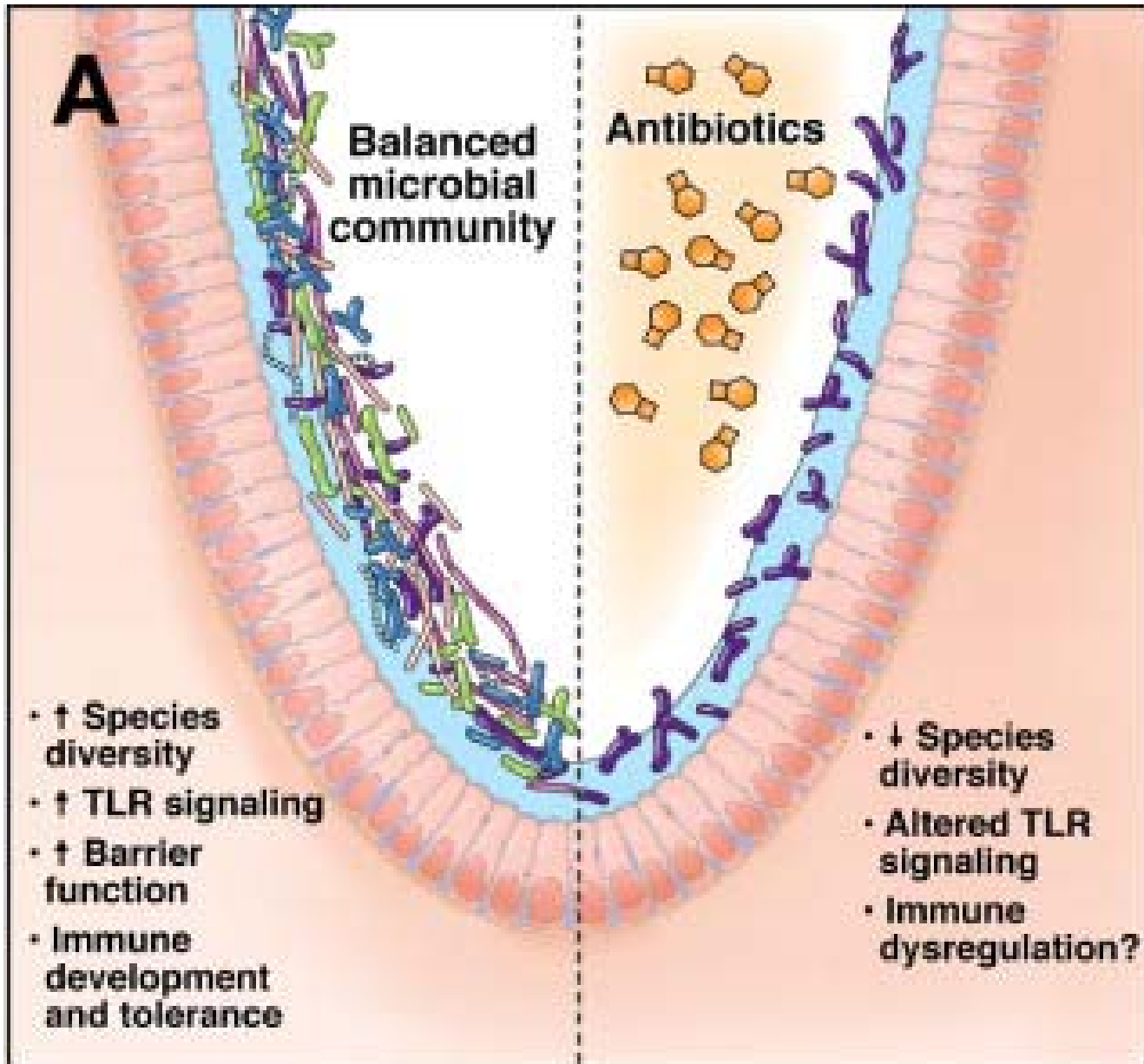
How can the new technologies
be exploited...

Microbial community

- Cultured vs uncultured
 - new agents causing/associated with disease
 - new association between pathogens
 - how does infection effect microbial flora
- Role of commensals in maintaining health
 - antibiotics/probiotics/prebiotics
- How does normal flora develop?

Variable Human Microbiome





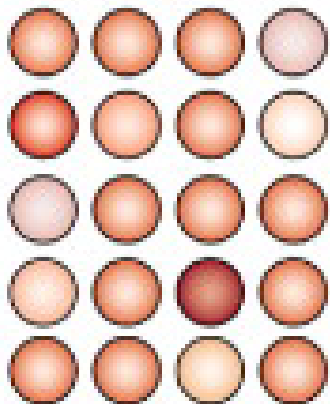
Antibiotics regulation

Prebiotics regulation

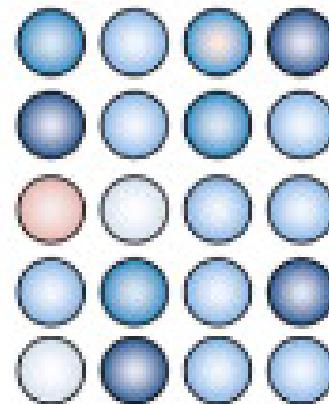
Probiotics regulation



Metagenome alteration



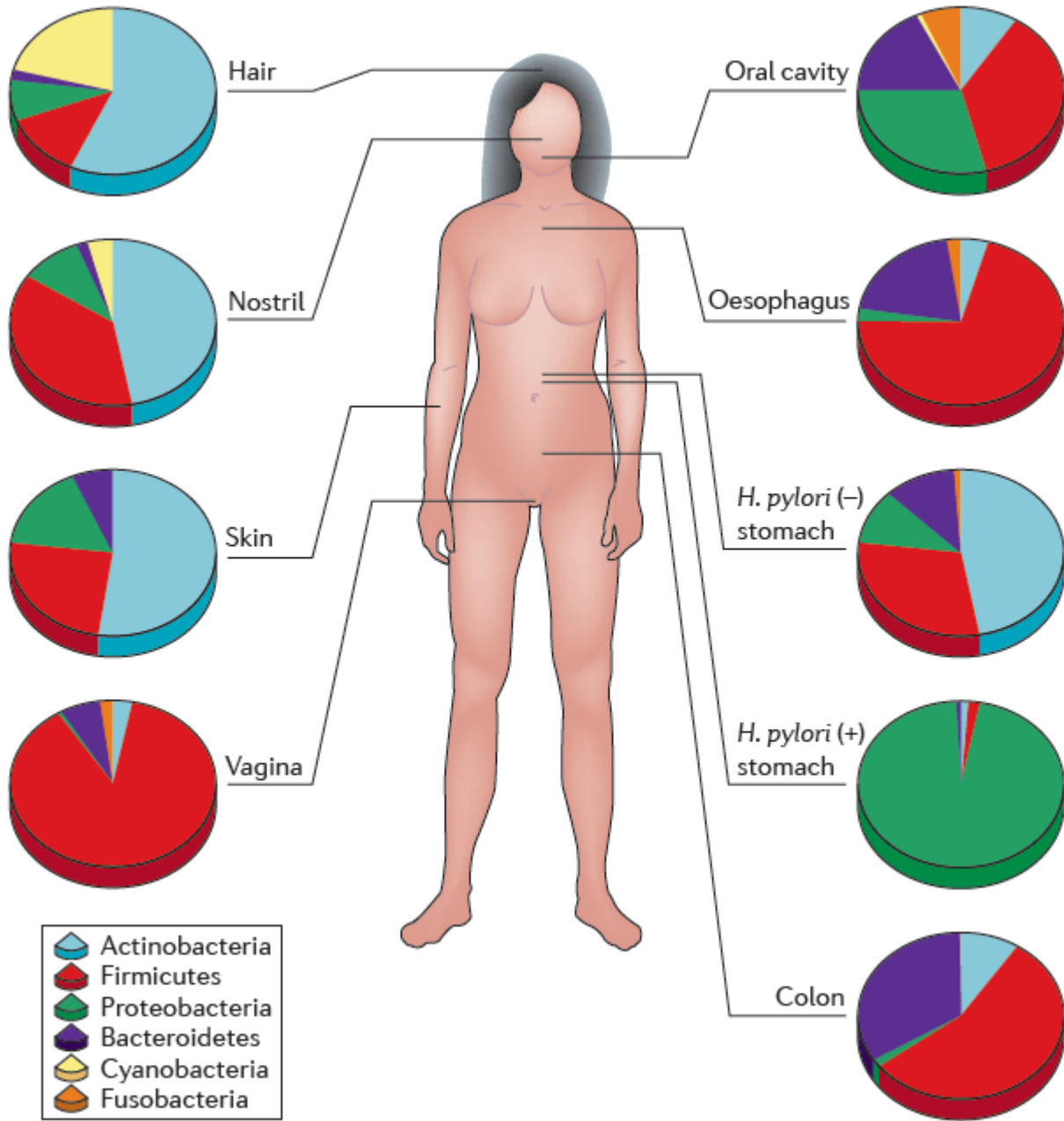
Diseased state



Healthy state

Metagenomics:

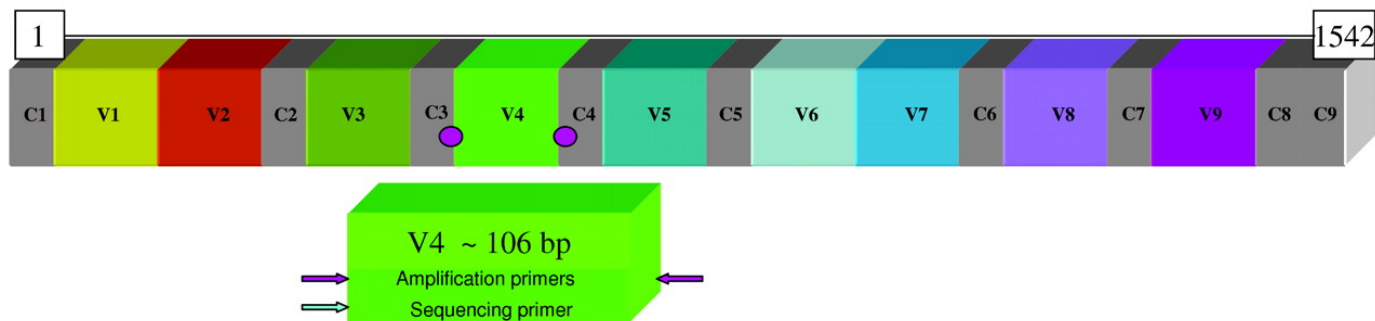
the culture-independent
genomics analysis of
microbial communities



Cho and Blaser (2012)
 Nature Reviews Genetics
 13:260-270

16S rRNA

- part of the ribosomal RNA
- a component of the small prokaryotic ribosomal subunit (30S)
- present in all bacteria
- contains conserved and variable regions

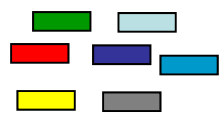




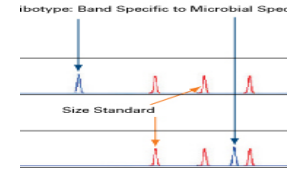
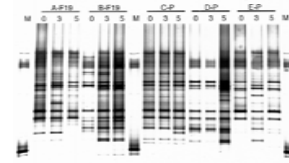
Genomic DNA



16s rRNA PCR



DGGE
T-RLFP



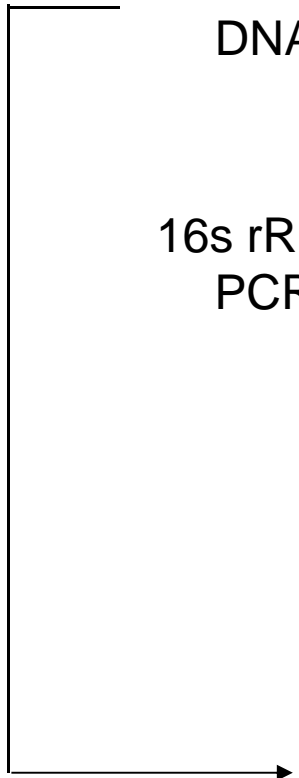
Array hybridisation



Library cloning

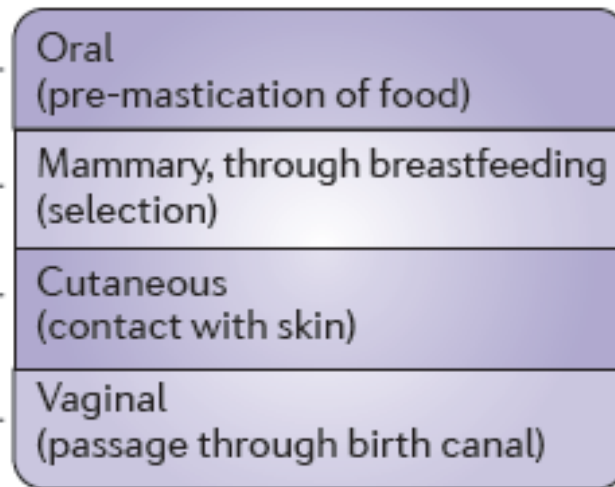
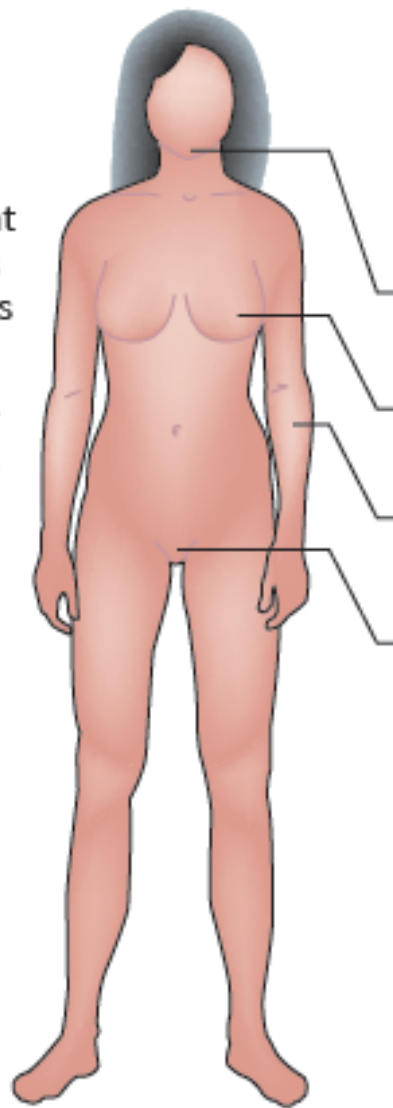
Sanger sequencing
(50-100 clones)

Sequencing
(454 or Illumina)



Effect of maternal exposures

- Environment
- Antisepsis
 - Antibiotics
 - Diet
- Other hosts
- Epigenetics



Dental amalgam

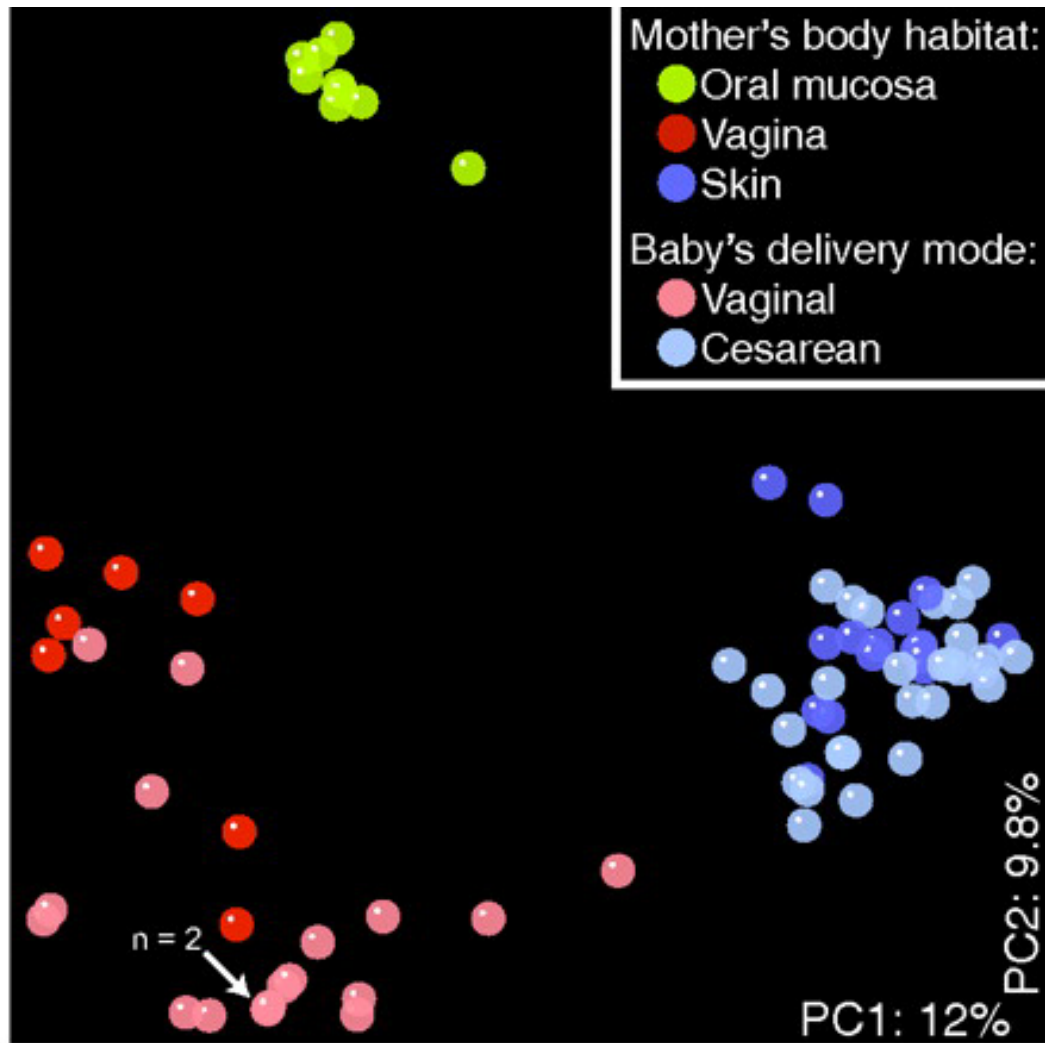
Bottle feeding



Early/
extensive
bathing

Caesarean section

Early-life
antibiotics

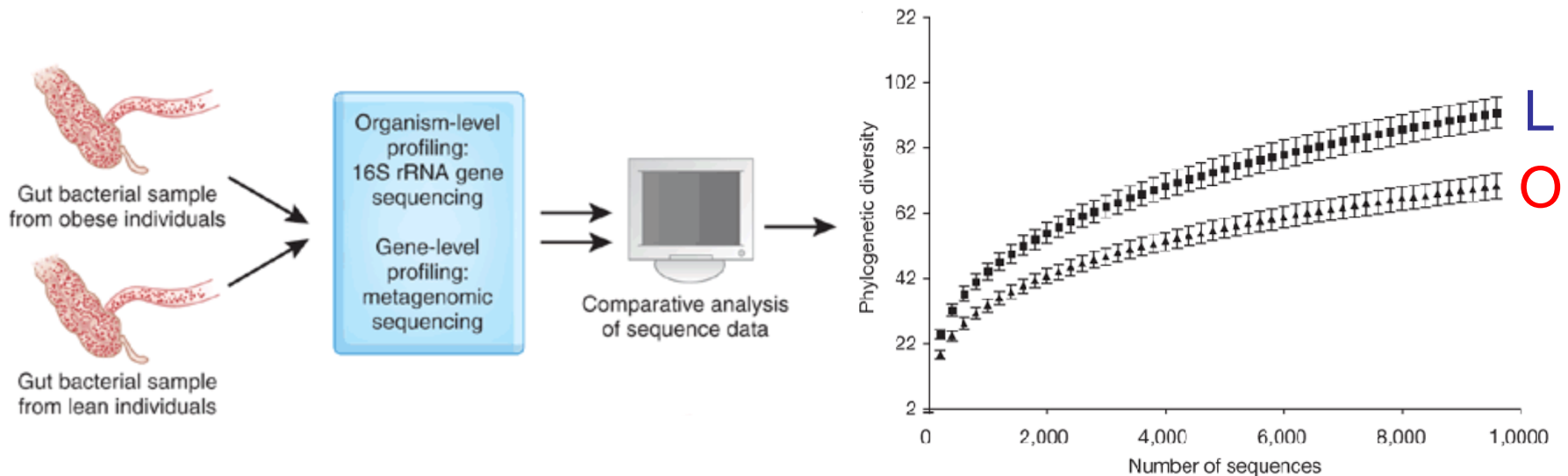


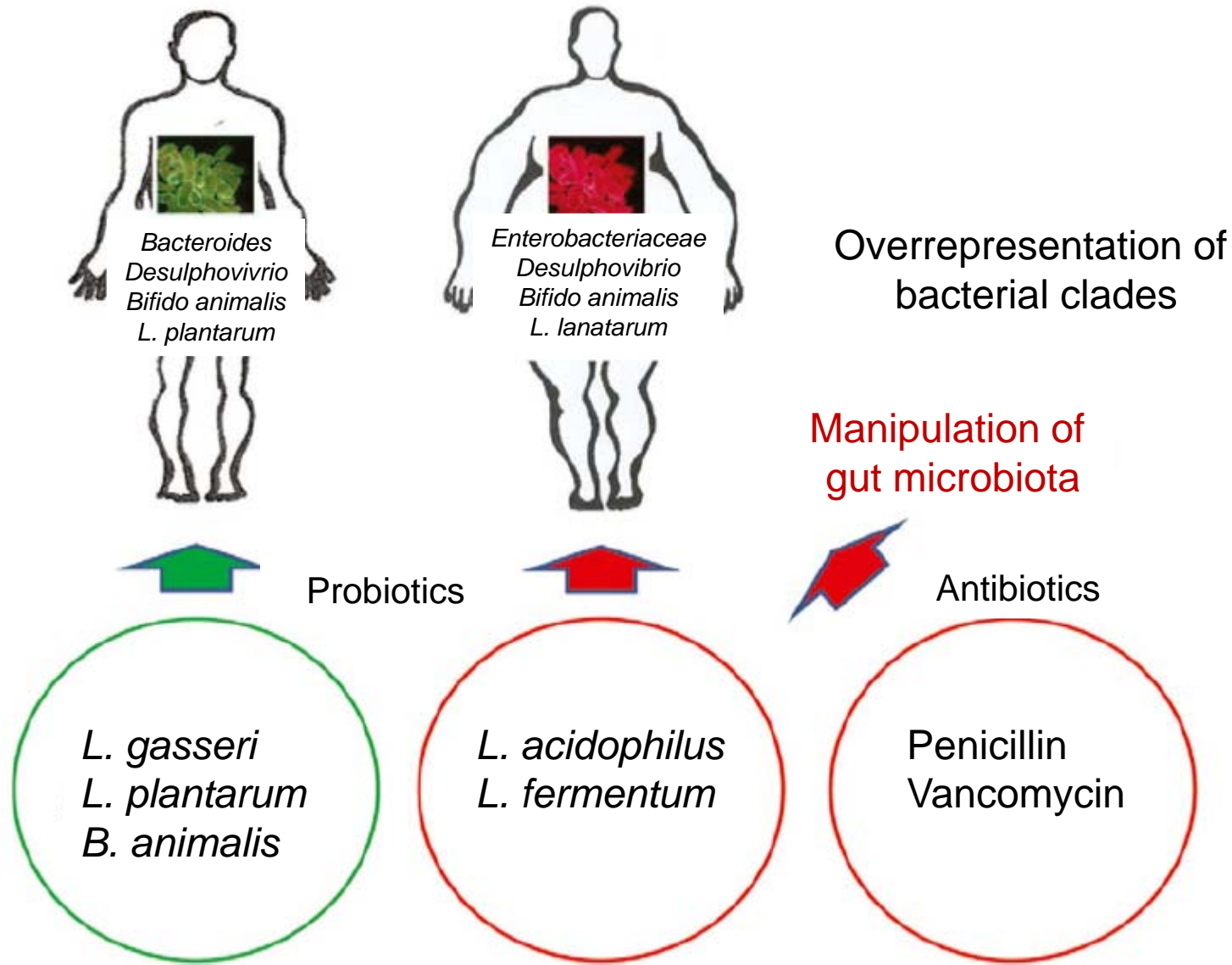
Dominguez-Bello MG et al. (2010) PNAS 107:11971-5

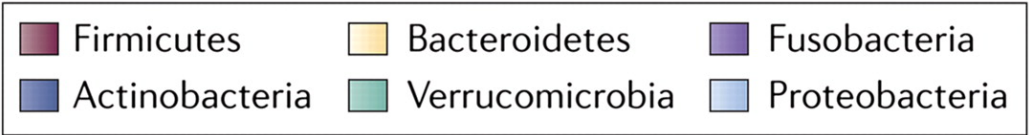
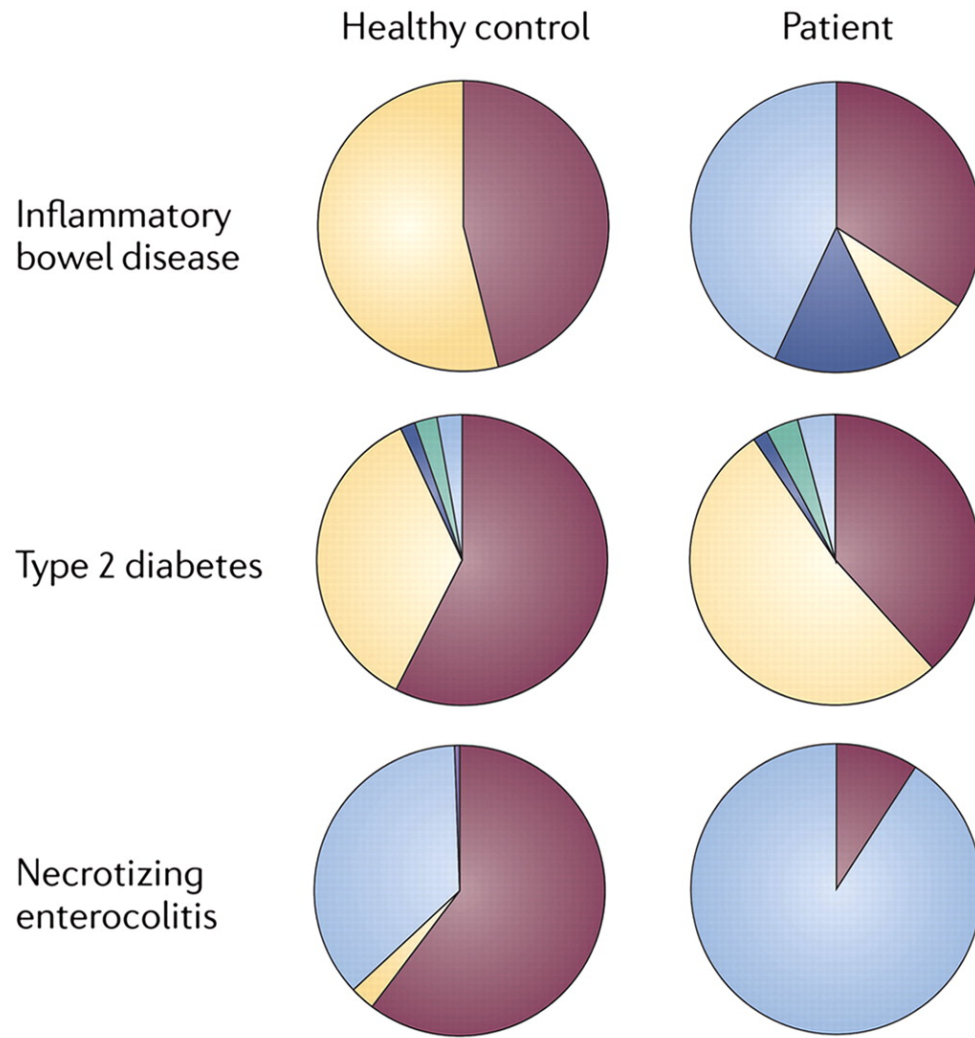
- Vaginally delivered infants acquired bacterial communities resembling their own mother's vaginal microbiota
- C-section infants harboured bacterial communities similar to those found on the skin surface

Turnbaugh et al. (2009) Nature 457, 480-484

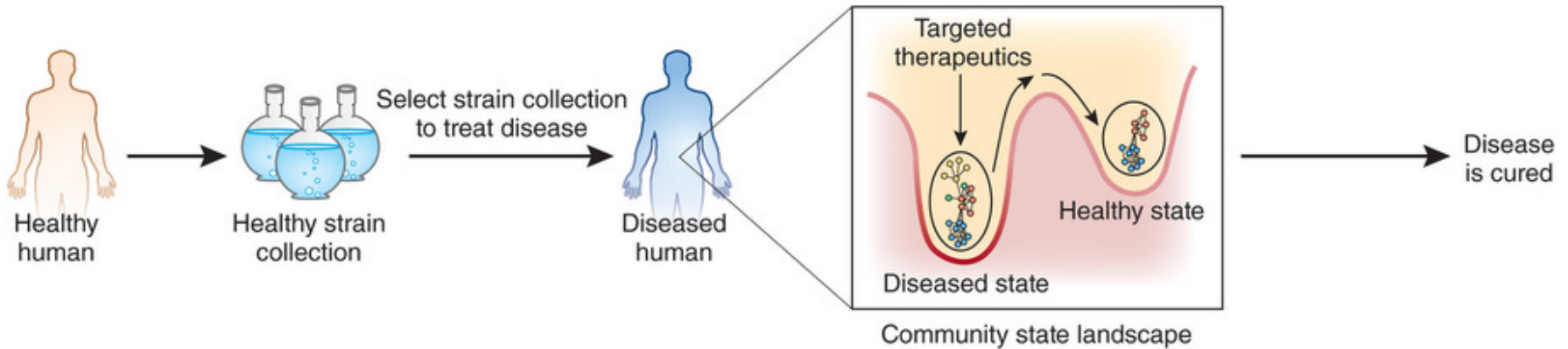
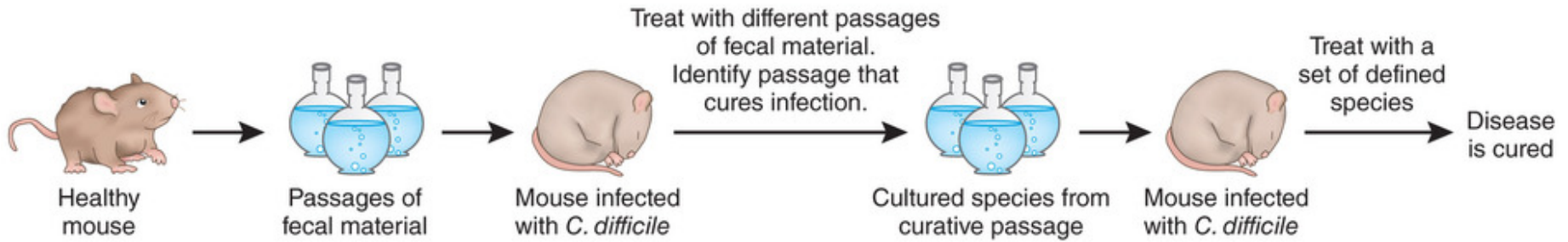
A core gut microbiome in obese and lean twins





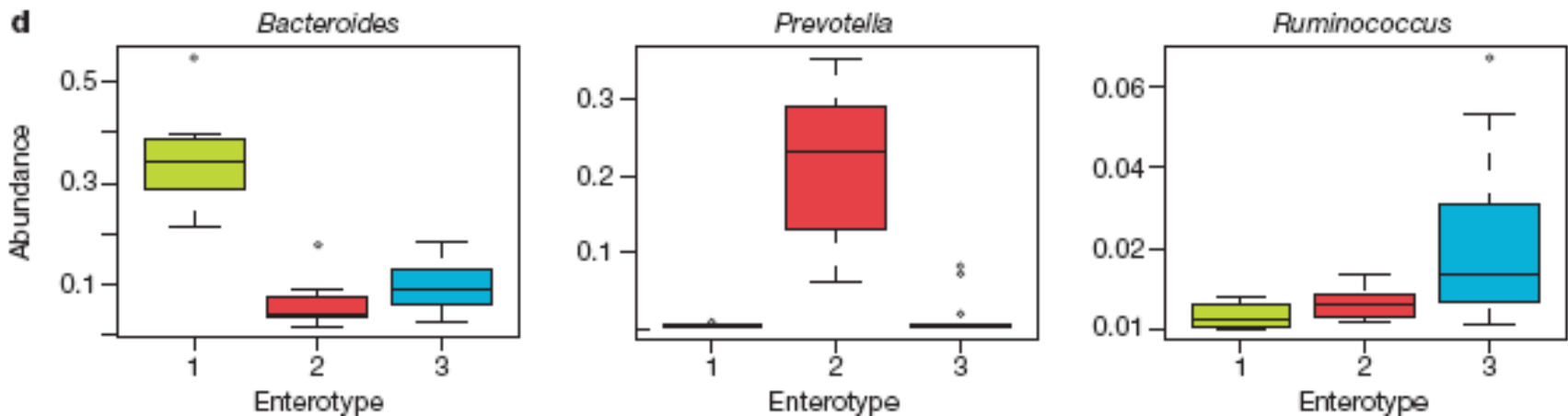
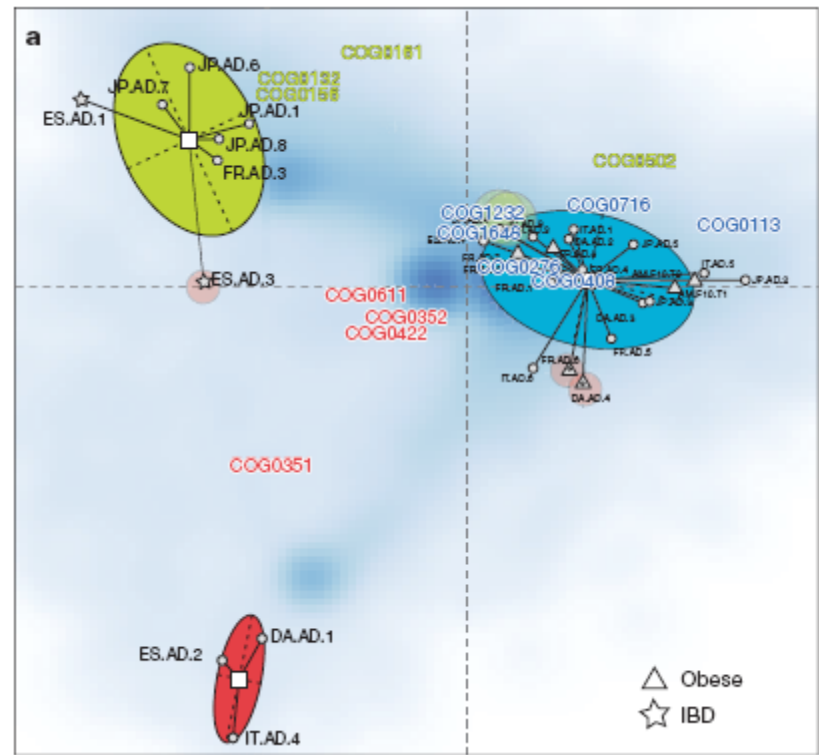


Lawley, T.D. *et al.* (2012) PLoS Pathog 8:e1002995



Relman *et al.* (2013) Nature Biotechnol 31:35-37.

Arumugam et al. (2011)
 Enterotypes of the human
 gut microbiome.
 Nature 473:174-180



- human gut microbiome is shared among family members
- each person's gut microbial community varies in the specific bacterial lineages present
- a wide array of shared microbial genes in individuals, comprising an identifiable 'core microbiome' at the gene but not the organism level
- obesity and some diseases are associated with phylum-level changes in the microbiota

Johnson CL and Versalovic J (2012)

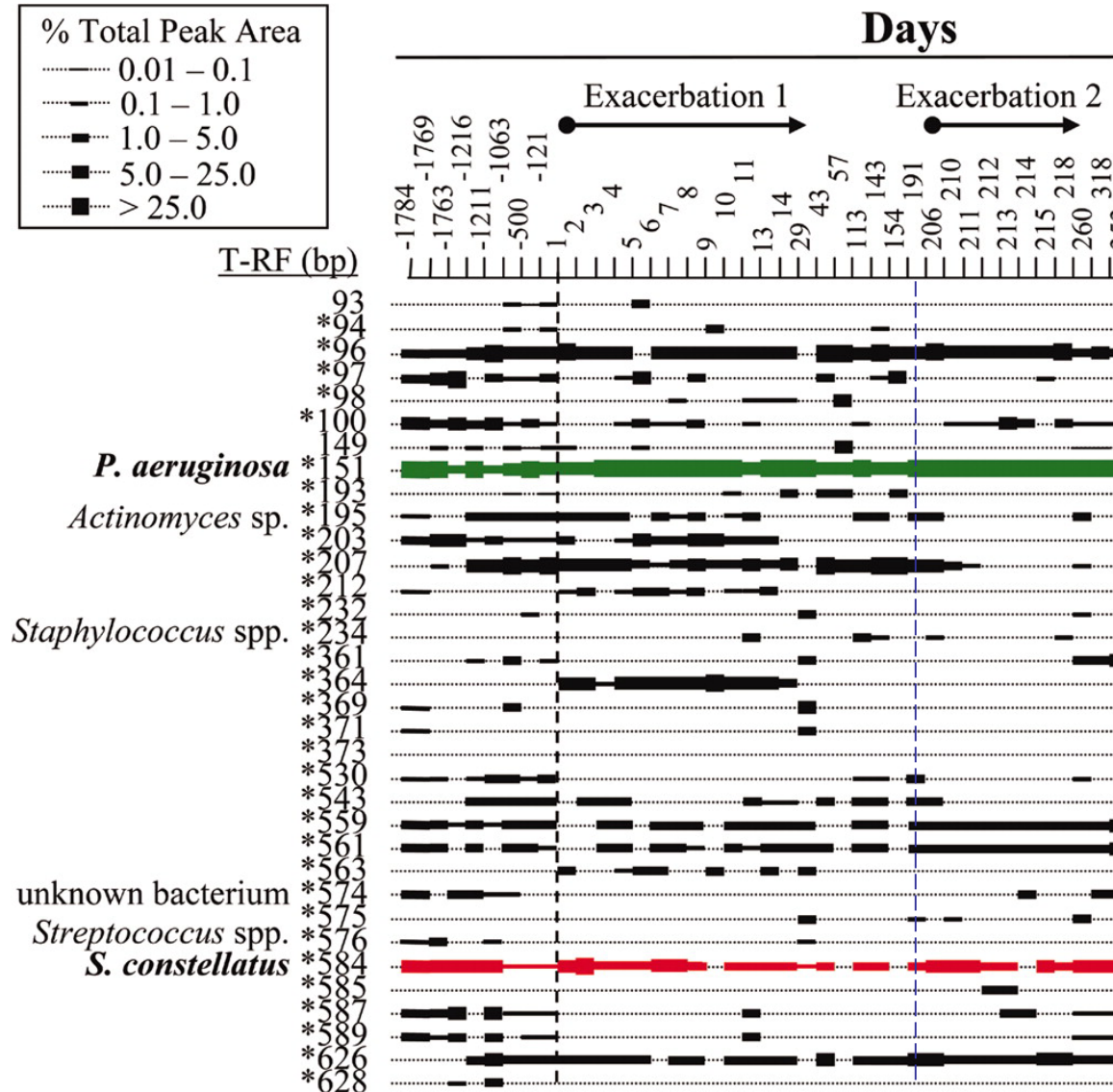
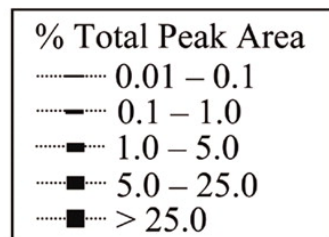
The human microbiome and its potential importance to pediatrics.

Pediatrics 129:950–96

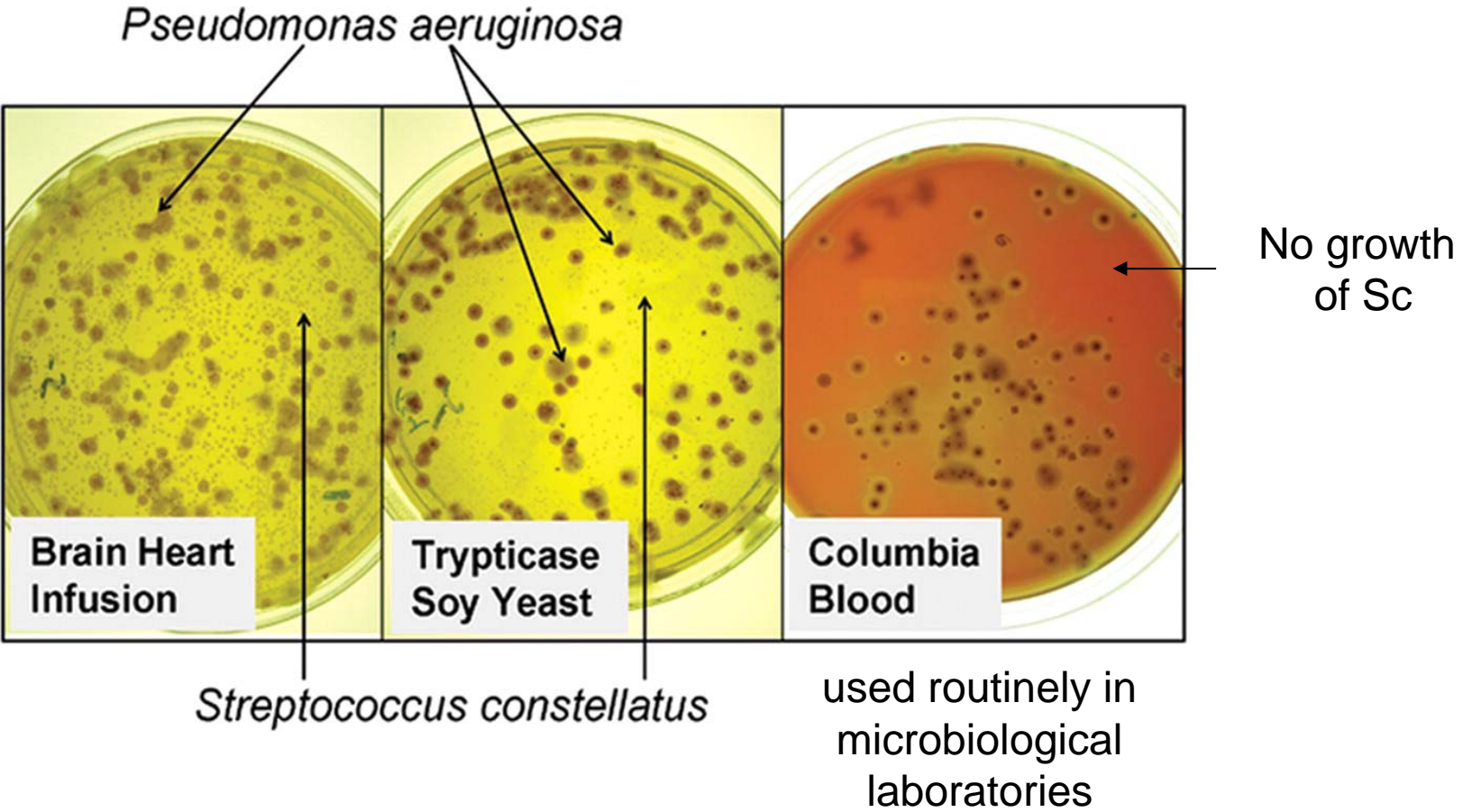
Cystic fibrosis (CF)

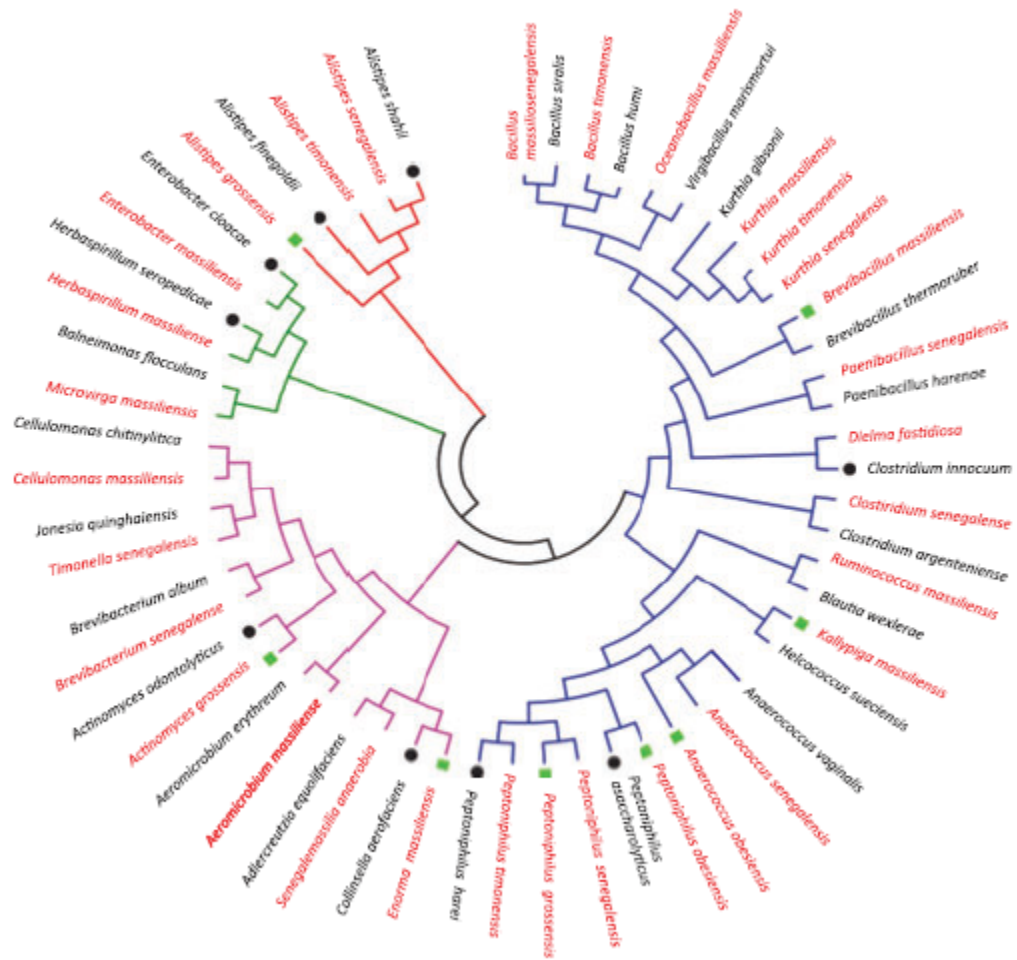
- Sibley CD et al. (2008) PNAS 105:15070-5
A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients
- T-RLFP analysis of individual CF patients

T-RFLP analysis of the microbial communities in sputum samples collected at admission to hospital for the first exacerbation and during the following year (days 1 to 368) and 5 retrospective samples (days -1784 to -121)



Why has *S. constellatus* been missed?

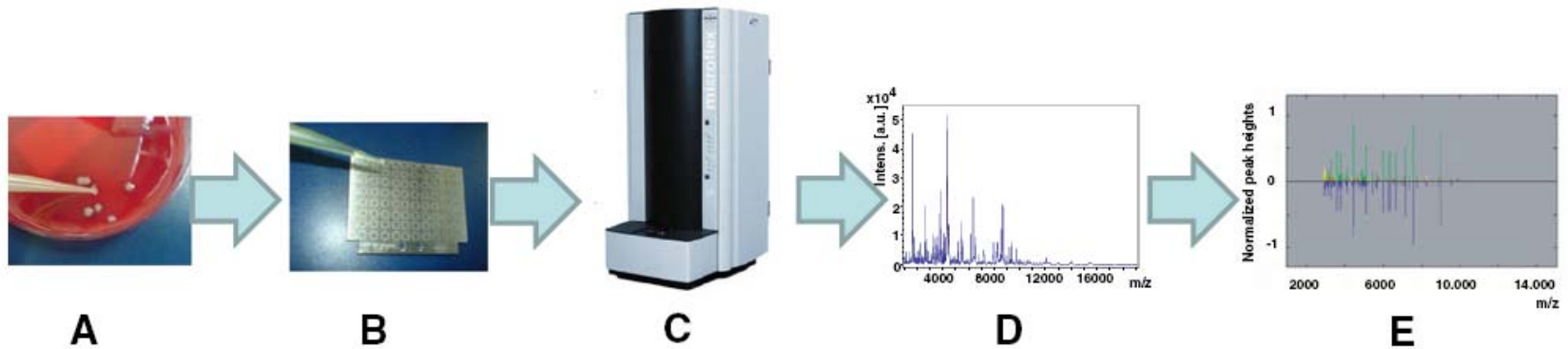




Culture (78 conditions)
 VS
 16S sRNA
 Stool samples
 (2 X Sengalese)
 1 X “French obese”

Red = new species (31)
 ● = obese only

Key technology: MALDI-TOF for the identification of bacteria



Wieser et al. (2012) Appl Microbiol Biotechnol 93:965-74

(a)

Culturomics

289

Pyrosequencing

416

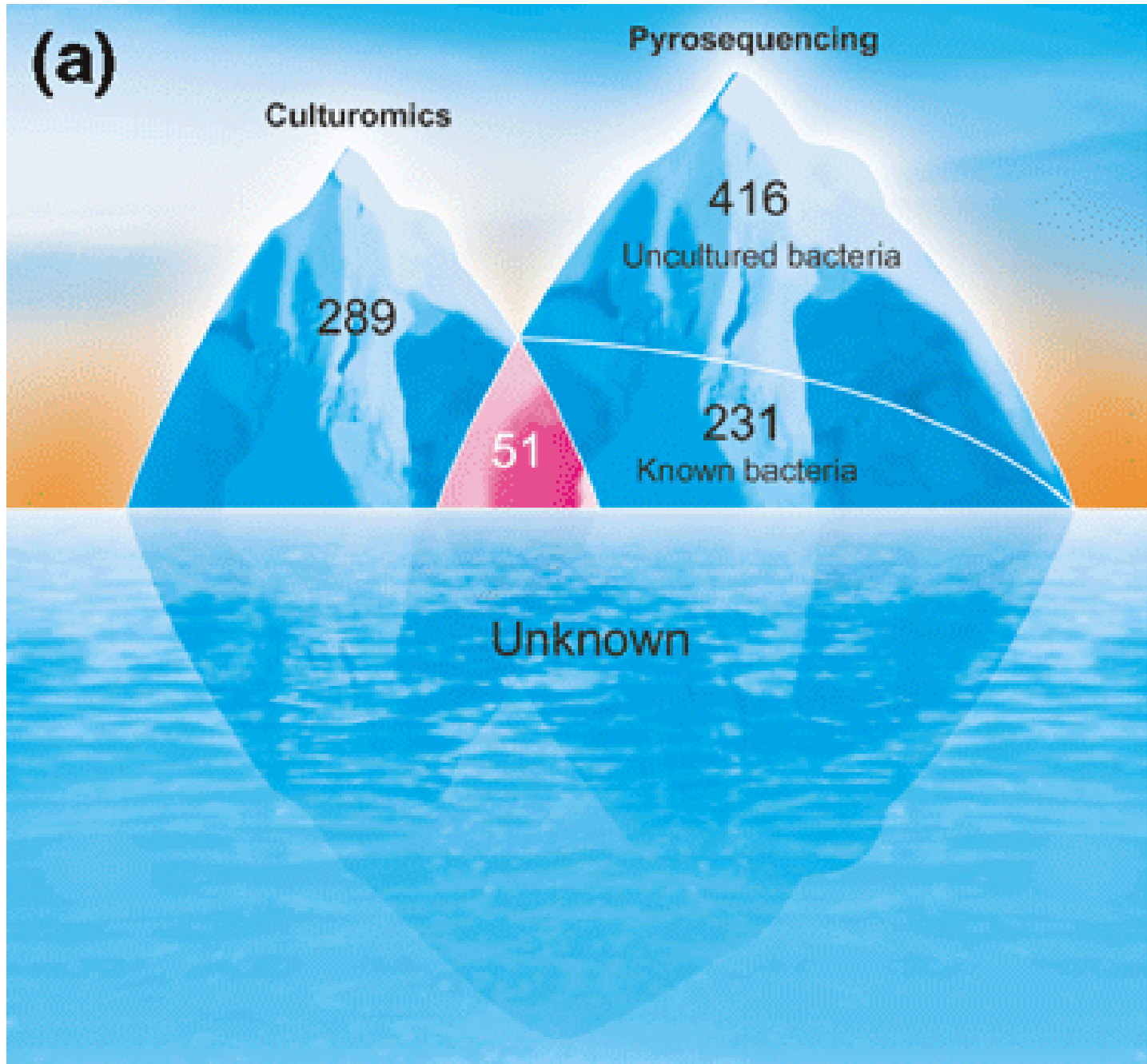
Uncultured bacteria

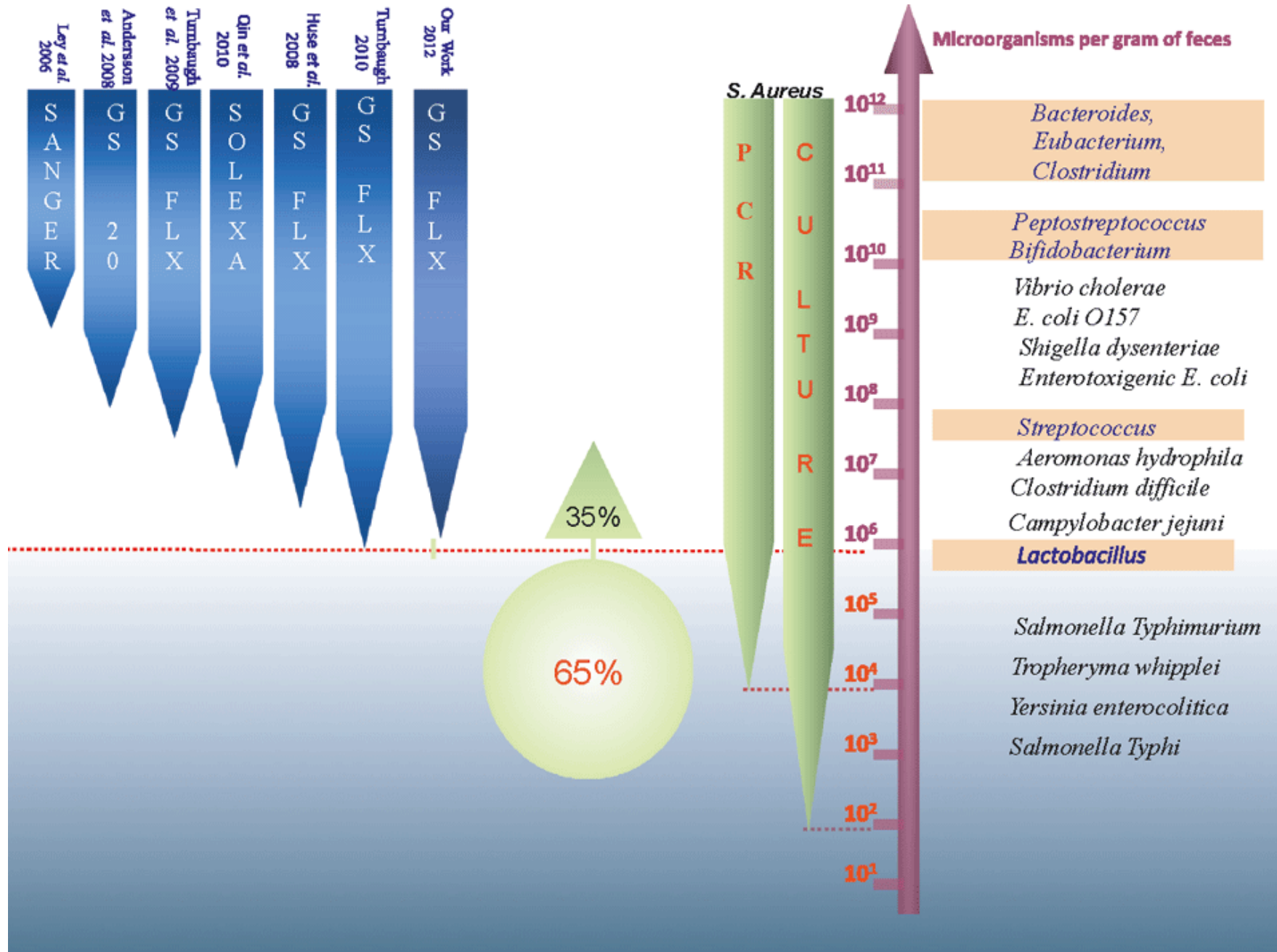
231

Known bacteria

51

Unknown





Identification of new pathogens

- Isolated RNA from brain, cerebrospinal fluid, serum, kidney, and liver from patients (kidney or liver transplant)
- RNA-seq → subtraction

Gustavo Palacios et al. (2008) A new Arenavirus in a cluster of fatal transplant-associated diseases. NEJM 358:991-998.

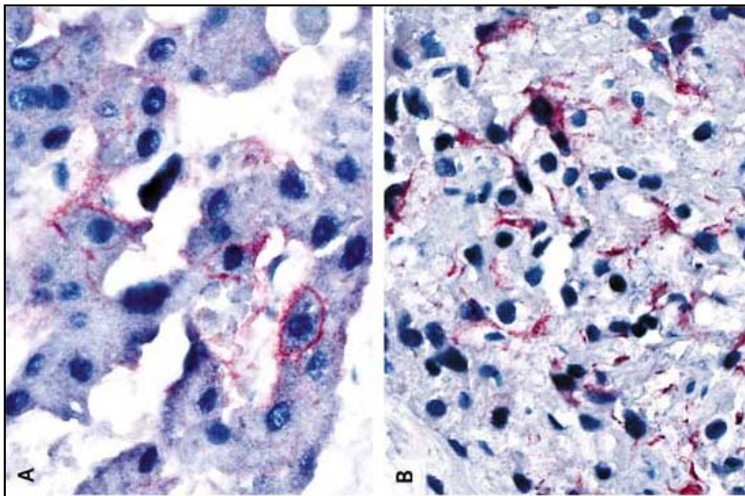
RNA → DNAase digestion → reverse transcribe to cDNA → 454 sequencing



confirm by culture
PCR, serology

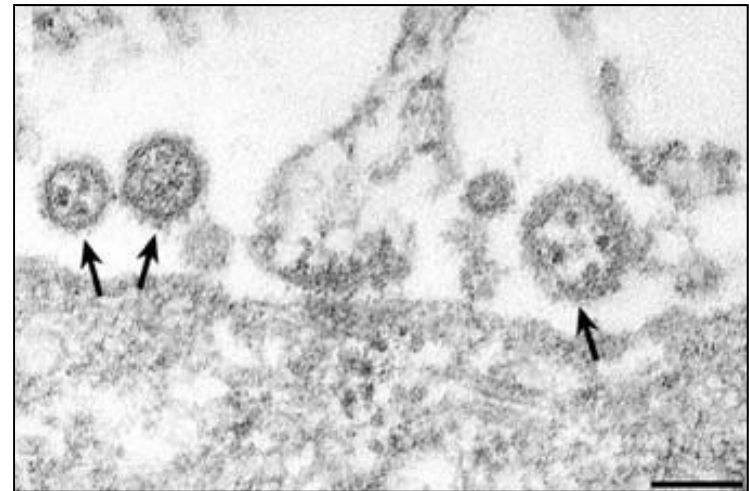
← identify new virus ←

remove known
human sequences



liver

kidney



Virus shedding from Vero E6 cells

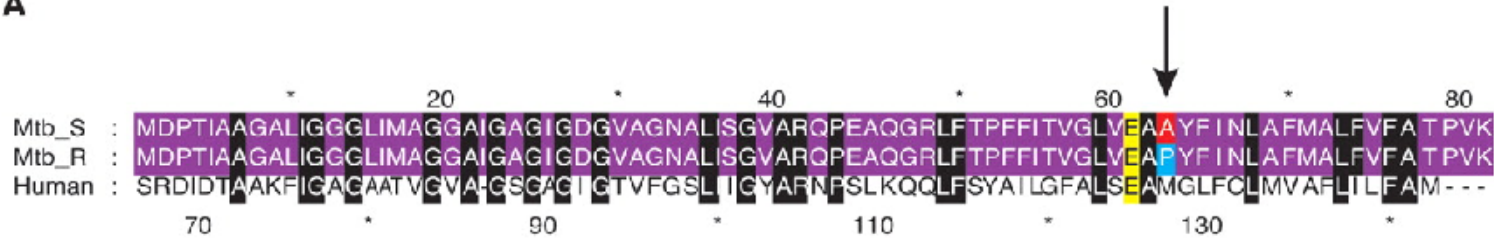
Other uses of HTS technology...

(1) Identifying target of new antibiotics

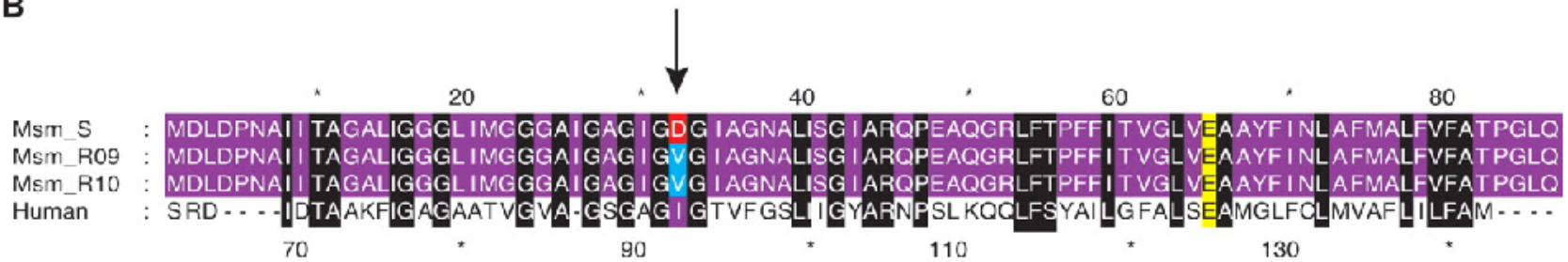
e.g. Andreis *et al.* (2005) *Science* 307:223–227

Generate mutants in vitro → WGS by 454 sequencing

A



B

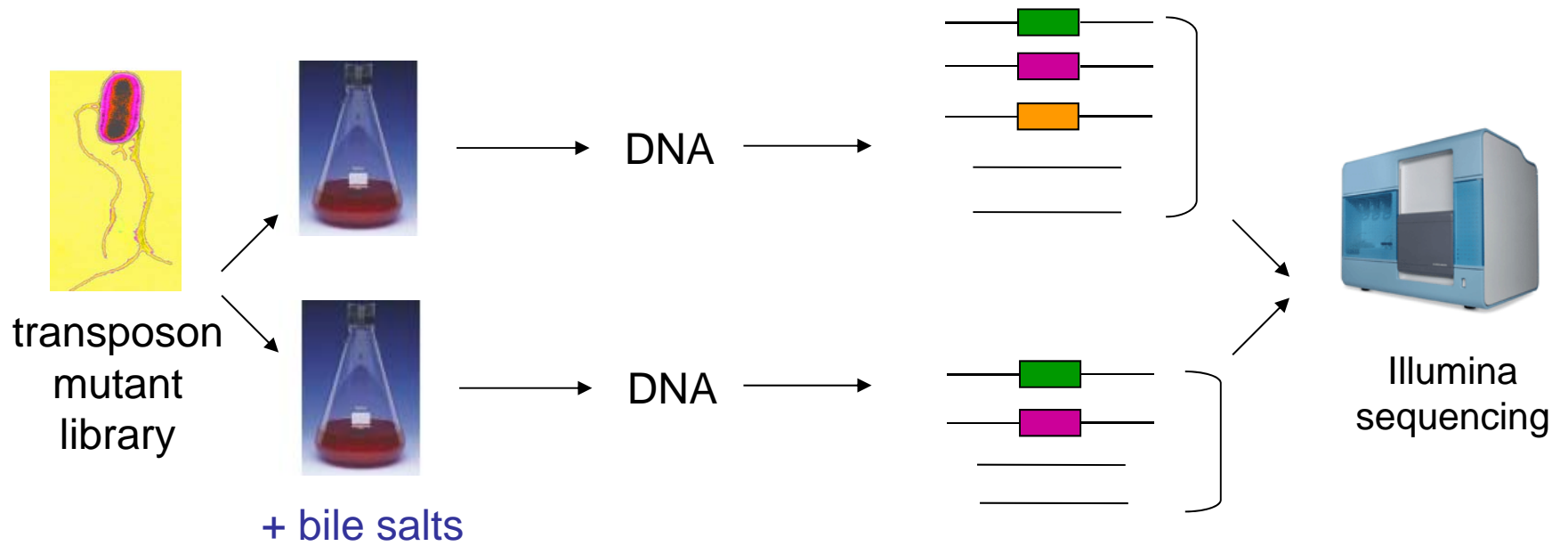


“Point mutations that conferred R207910 resistance were identified by comparative analysis of the genome sequences of susceptible and resistant strains of *M. tuberculosis* and *M. smegmatis*. The **only** gene commonly affected in all three independent mutants encodes *atpE*, a part of the F0 subunit of ATP synthase. This finding indicates that R207910 inhibits the proton pump of *M. tuberculosis* ATP synthase.”

(2) Identification of essential genes

- Transposon Directed Insertion site Sequencing (TraDIS)
- *Salmonella* Typhi
 - 22 million people are infected/annum
 - 220,000 die/annum

Langridge et al. (2009) Simultaneous assay of every *Salmonella* Typhi gene using one million transposon mutants. *Genome Res* 19:2308-16.

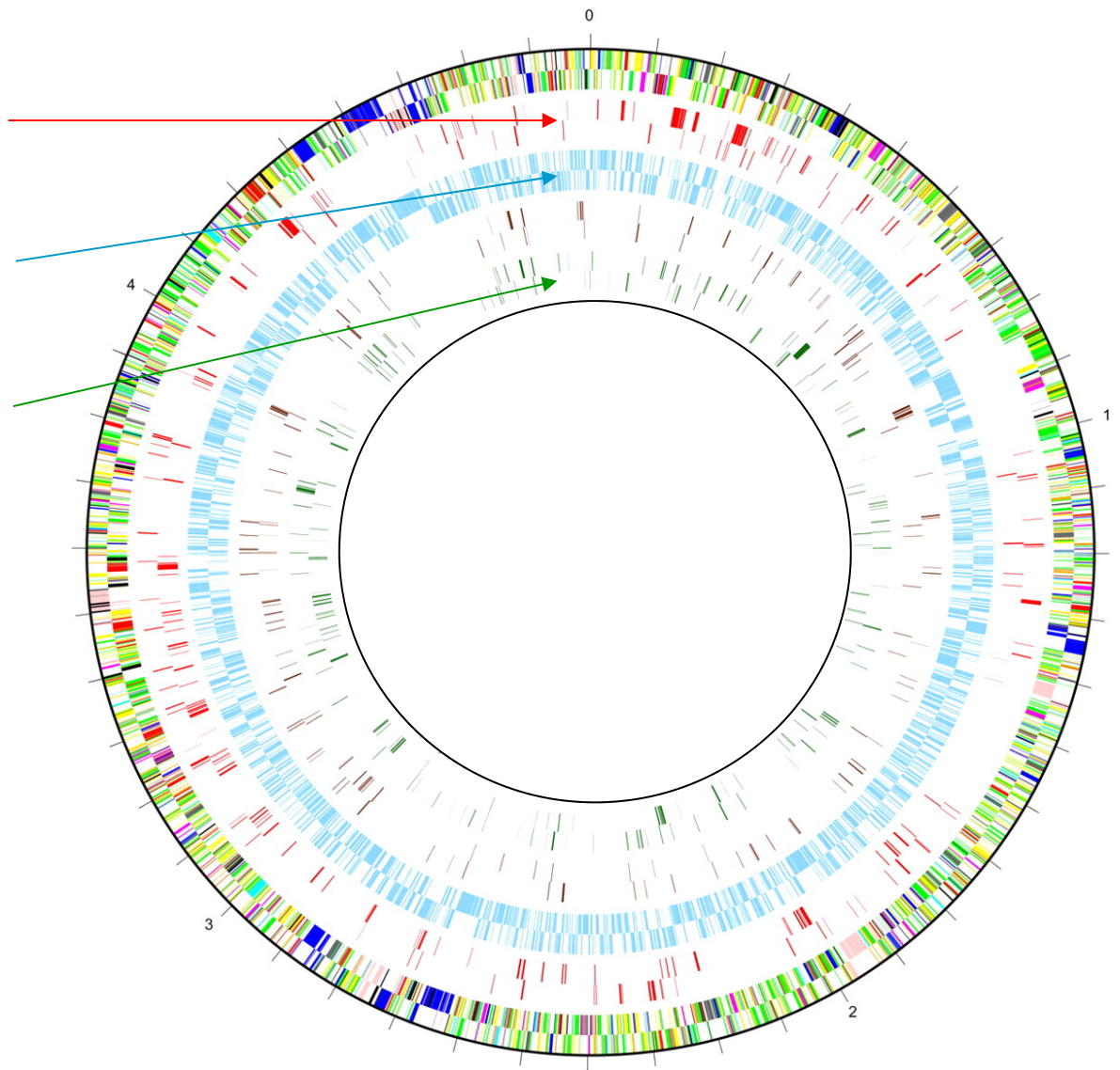


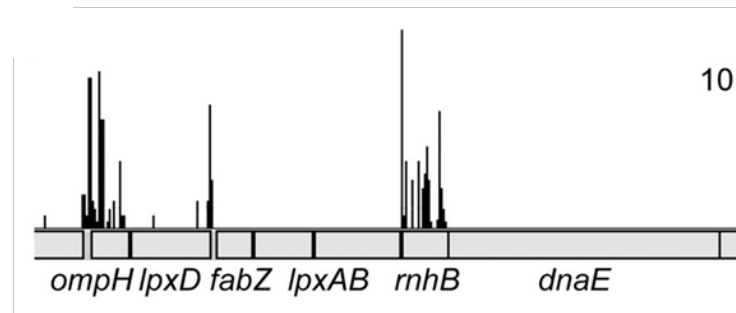
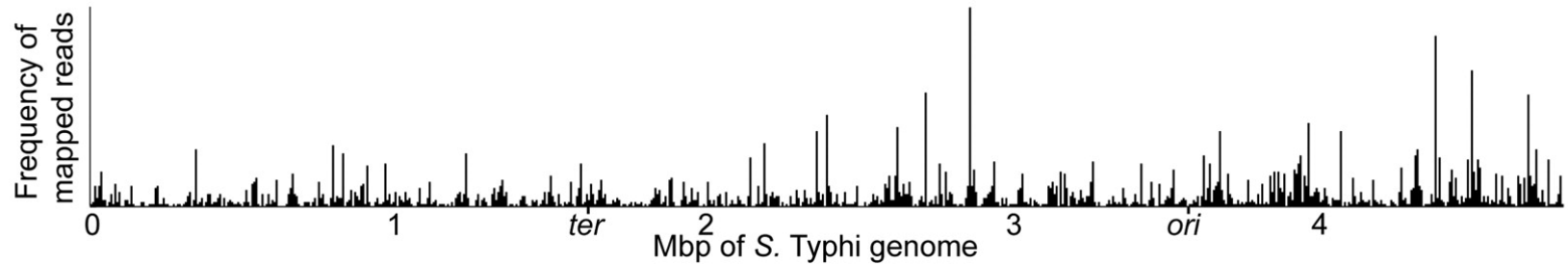
Loss of a mutant = essential for growth in that condition

Red = essential

Blue = non-essential

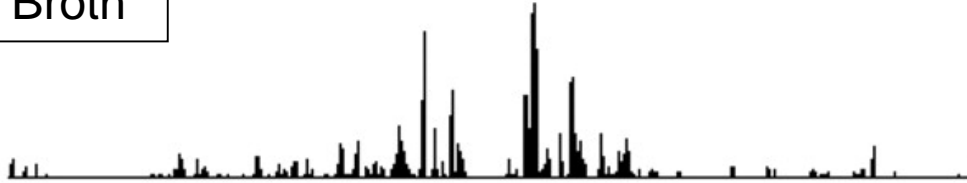
Green = essential for growth in bile salts



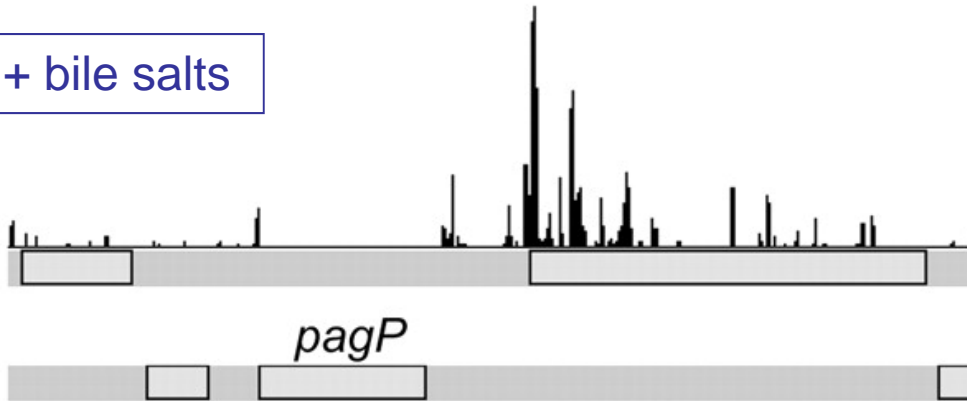
A

In broth: *S. Typhi* needs only **356** genes for survival
(4162 genes were not essential)

Broth

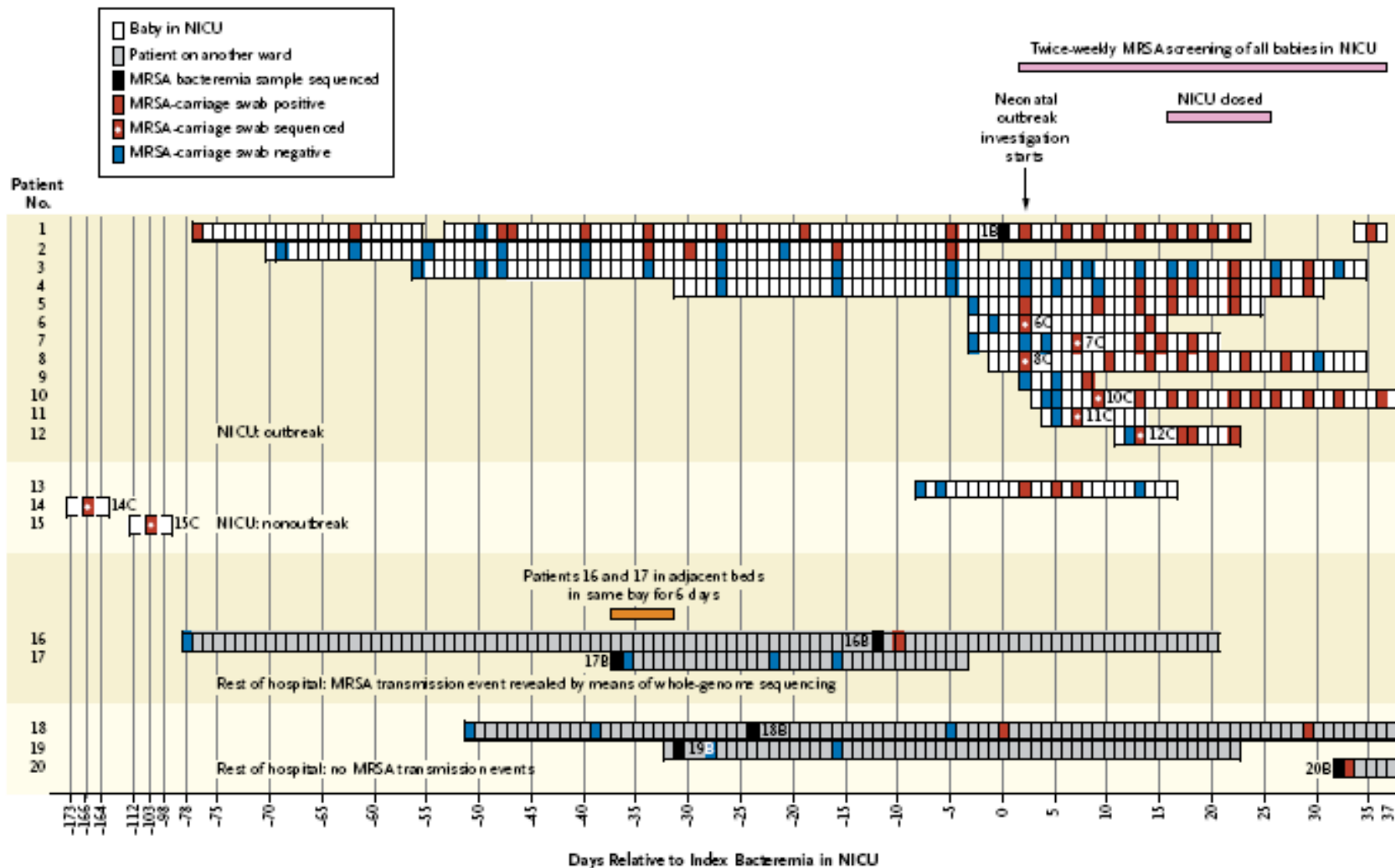


+ bile salts



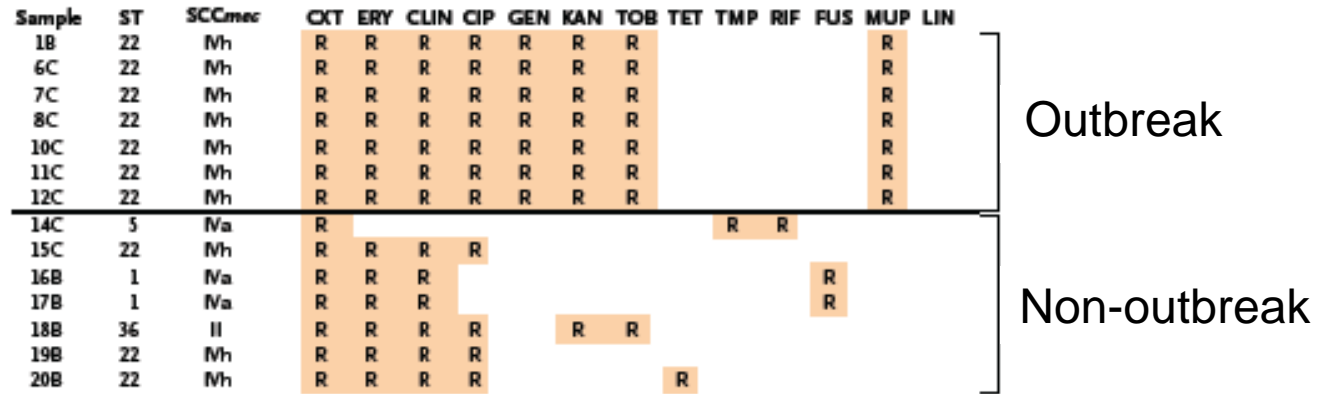
pagP is an essential gene for growth in bile salts

In infectious disease outbreaks...

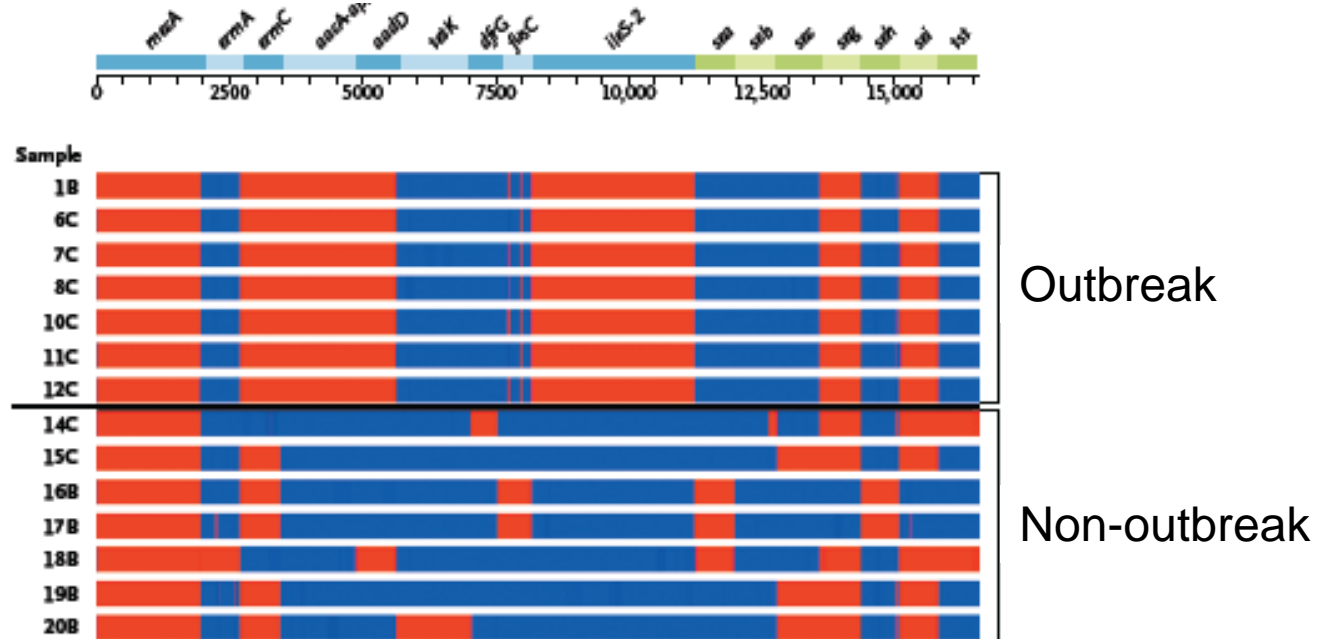


Köser CU et al. (2012) NEJM 366:2267-75.

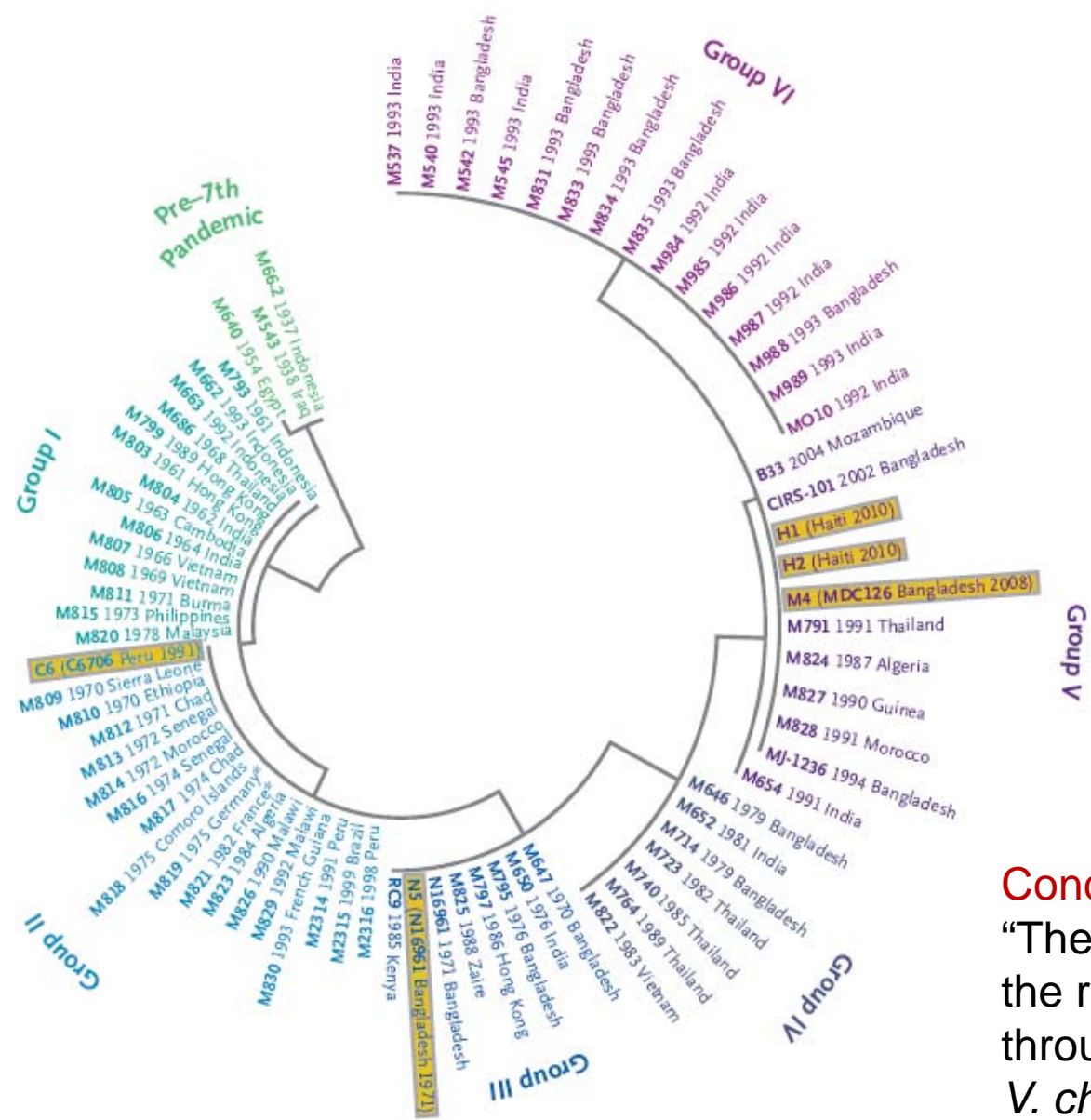
Antibiograms



Resistome and toxome



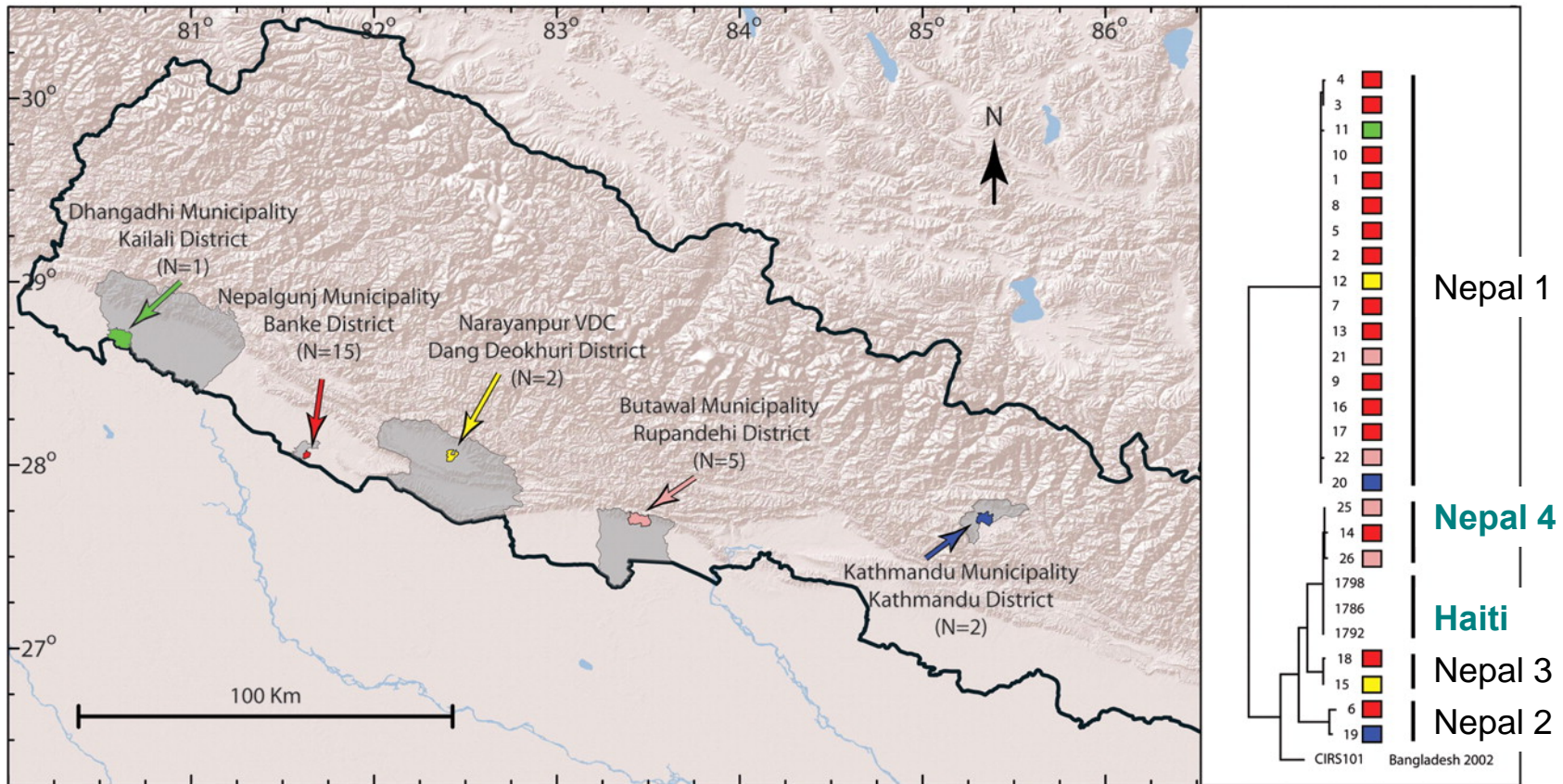
Chin CS et al. NEJM (2011) 364:33-42.



Conclude

“The Haitian epidemic is probably the result of the introduction, through human activity, of a *V. cholerae* strain from a distant geographic source.”

Nepal locations where the *V. cholerae* O1 Ogawa strains were isolated



Hendriksen R S et al. (2011) mBio 2:e00157-11.

E. coli HUS (Germany)...

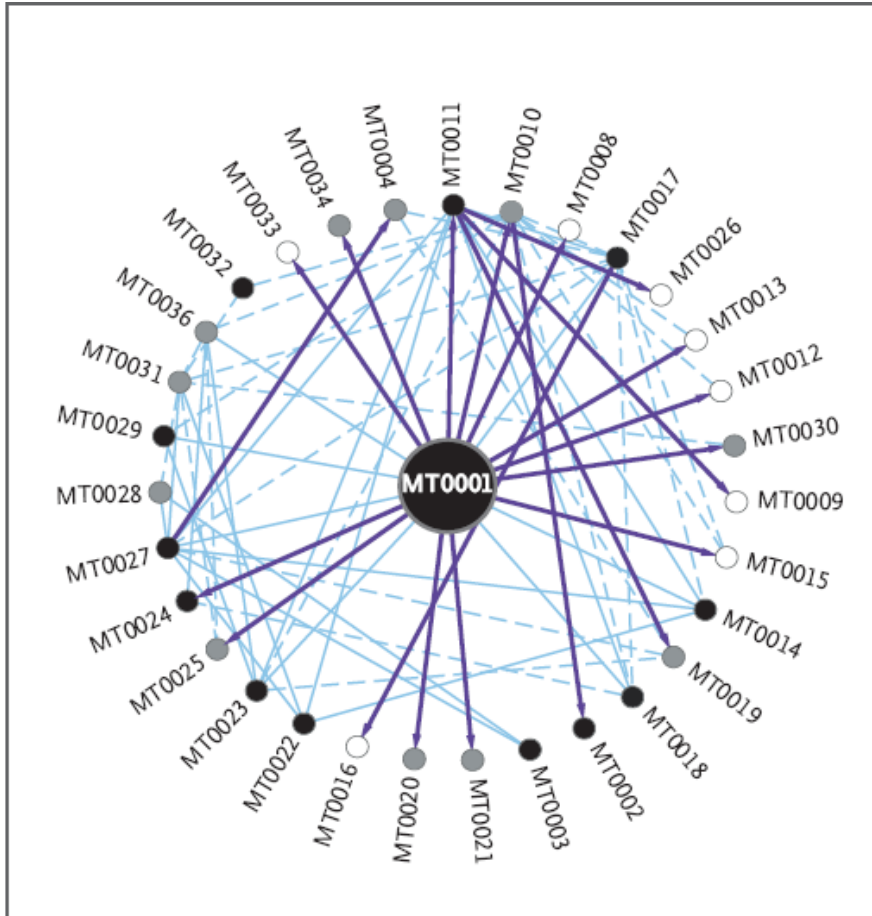


<http://www.nejm.org/doi/full/10.1056/NEJMoa1106920>

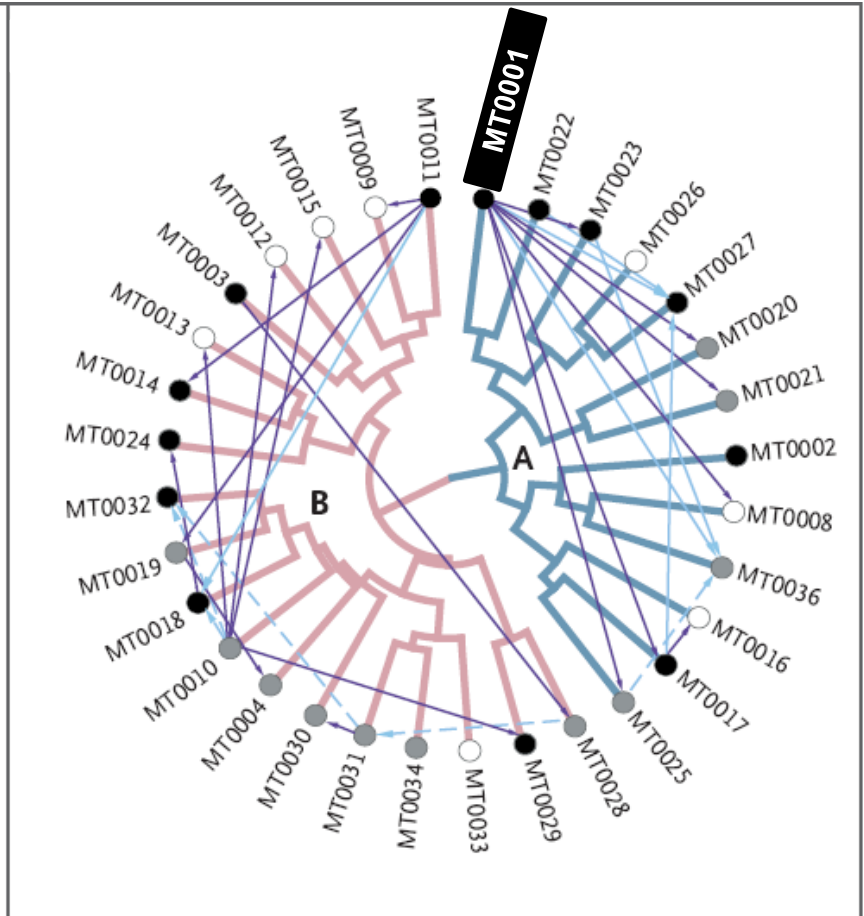
Conclude

Our findings suggest that horizontal genetic exchange allowed for the emergence of the highly virulent Shiga-toxin–producing enteroaggregative *E. coli* O104:H4 strain that caused the German outbreak.

VNTR and Social Network Analysis



WGS and Social-Network Analysis



Gardy et al. (2011) Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. NEJM 364:730-9.

Some thoughts...

- Cost
- Bioinformatic overload
- Translation to the clinic
- Use in paediatrics =
limited by your imagination

Some other references

Lee YK and Mazmanian SK (2010) has the microbiota played a critical role in the evolution of the adaptive immune system. *Science* 330:1768-1773.

Petrosino JF et al. (2009). Metagenomic pyrosequencing and microbial identification. *Clin Chem* 55:856-66.

Reid R et al. (2011) Microbiota restoration: natural and supplemented recovery of bacterial communities. *Nature Microbiol Revs* 9:27-38

Voelkerding KV et al (2009) Next-generation sequencing: from basic research to diagnostics. *Clin Chem* 55:641-58.