Advancing the analysis of gut microbiome in critically ill patients using Hi-C technology

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Human gut microbiome in intensive care patients

In critically ill subjects, gut dysbiosis leads to inflammation, tissue injury – and back in a ”vicious circle”.

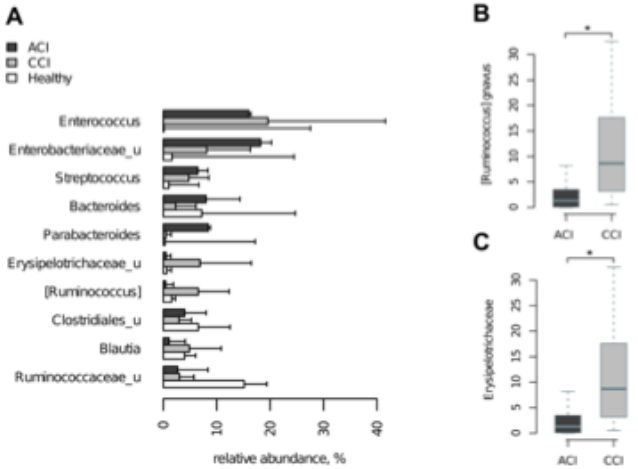
Microbiome - a reservoir for virulent multidrug-resistant nosocomial pathogens.

Chronically critically ill (CCI) patients:

- long stay in ICU
- regular treatment with multiple classes of antibiotics
- inflammation
- myopathy
- increased susceptibility to infection
- metabolic changes (hypercatabolism)
- hormonal changes
- cognitive impairment

Relevance to the COVID-19:

- ~7% of patients get bacterial co-infection.
- Some agents might come from the gut.
- Virulence potential of HGM should be explored.



Chernevskaya et al, Critical Care, 2020

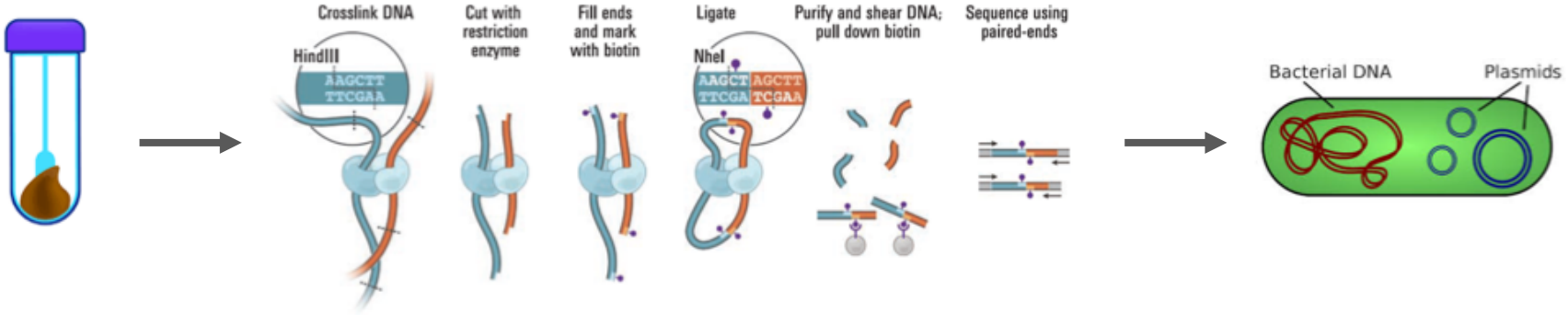
Hi-C metagenomics

The chromosome interaction signal is stronger:

- locally within a bacterial chromosome
- within a cell than between the cells.

Improved:

- genome assembly
- localization of mobile genetic elements



Lieberman-Aiden et al, 2009

Study design

Pilot set:
2 chronically critically ill patients x
2 time points

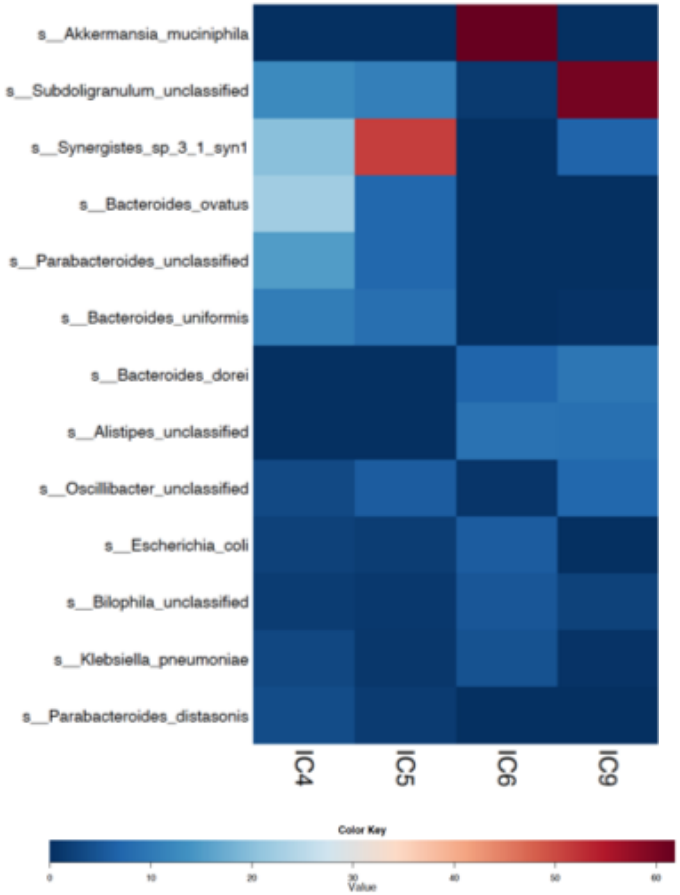
Sample type: stool.

Method: WGS + Hi-C sequencing (2 x 150 bp, Illumina).

	Patient A (samples 4 - 5)	**Patient B (samples 6 - 9)**
Age/Gender	75 / F	74 / M
Diagnosis	intracerebral hemorrhage	ischemic stroke
Length of stay in ICU, w	8	6
Support	Mechanical ventilation, enteral tube feeding (high calorie, low-residue)	
Timepoint 1	suspected bacterial infection pathogens in trachea (cultivation) pneumonia signs from CT Scan	
Timepoint 2	+1 week, negative clinical dynamics	+2 weeks, positive clinical dynamics

Basic analysis of gut community structure: pronounced dysbiosis

Timepoints: 4-5, 6-9.



WGS: decreased diversity driven by *Bacteroidaceae* and various opportunists (*Klebsiella*, *Escherichia*, *Proteus*, *Bilophila*).

MetaPhlan2

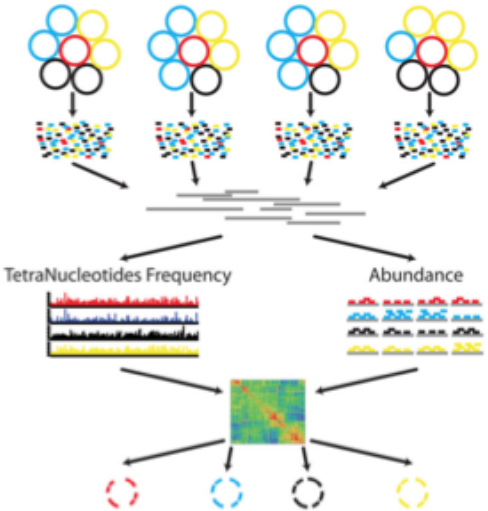
Metagenome-assembled genomes (MAGs)

From short NGS reads to microbes: contigs are binned into MAGs, basing on various principles.

Major MAG quality measures: # of MAGs/sample, completeness (%) and contamination (%).

WGS-MAGs

(MetaBat2 [Kang et al, 2015])

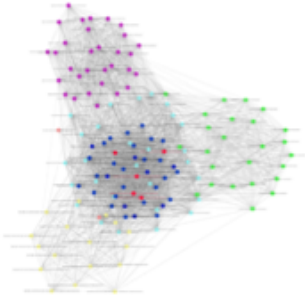


Hi-C-MAGs

(bin3c [DeMaere et al, 2019])

Graph: Hi-C contacts (edges) between WGS contigs (vertices).

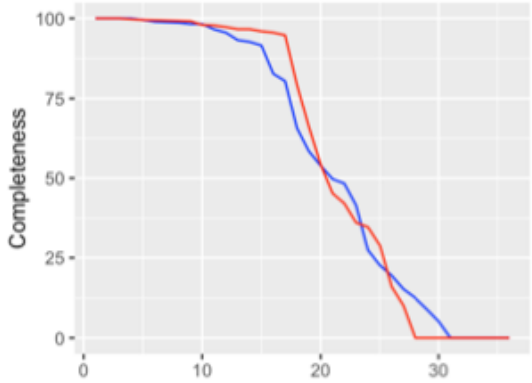
Identify the clusters.

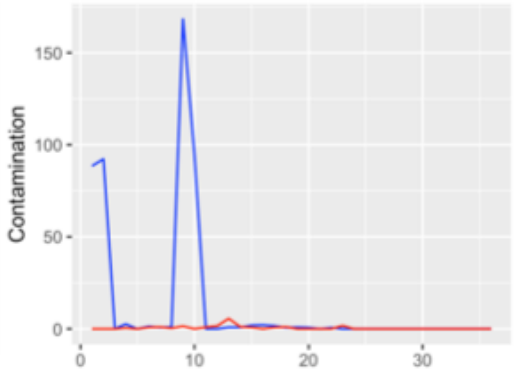


Hi-C allows to obtain higher quality MAGs compared to WGS

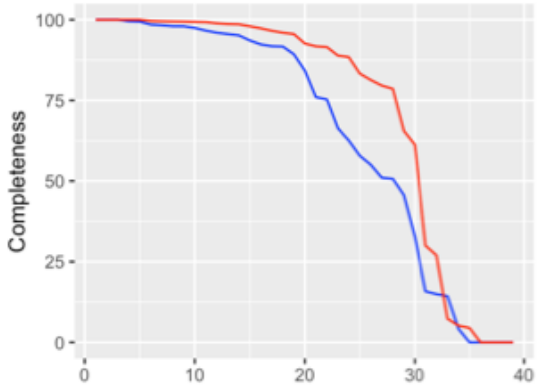
Hi-C
WGS

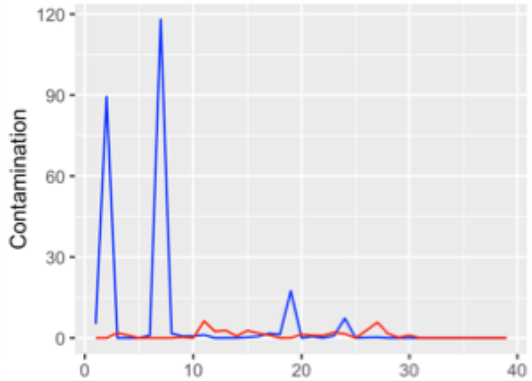
Sample 6





Sample 9





CheckM

~20 high-quality MAGs per sample

Number of high-quality MAGs (completeness > 80%) is similar.

Completeness is higher for Hi-C.

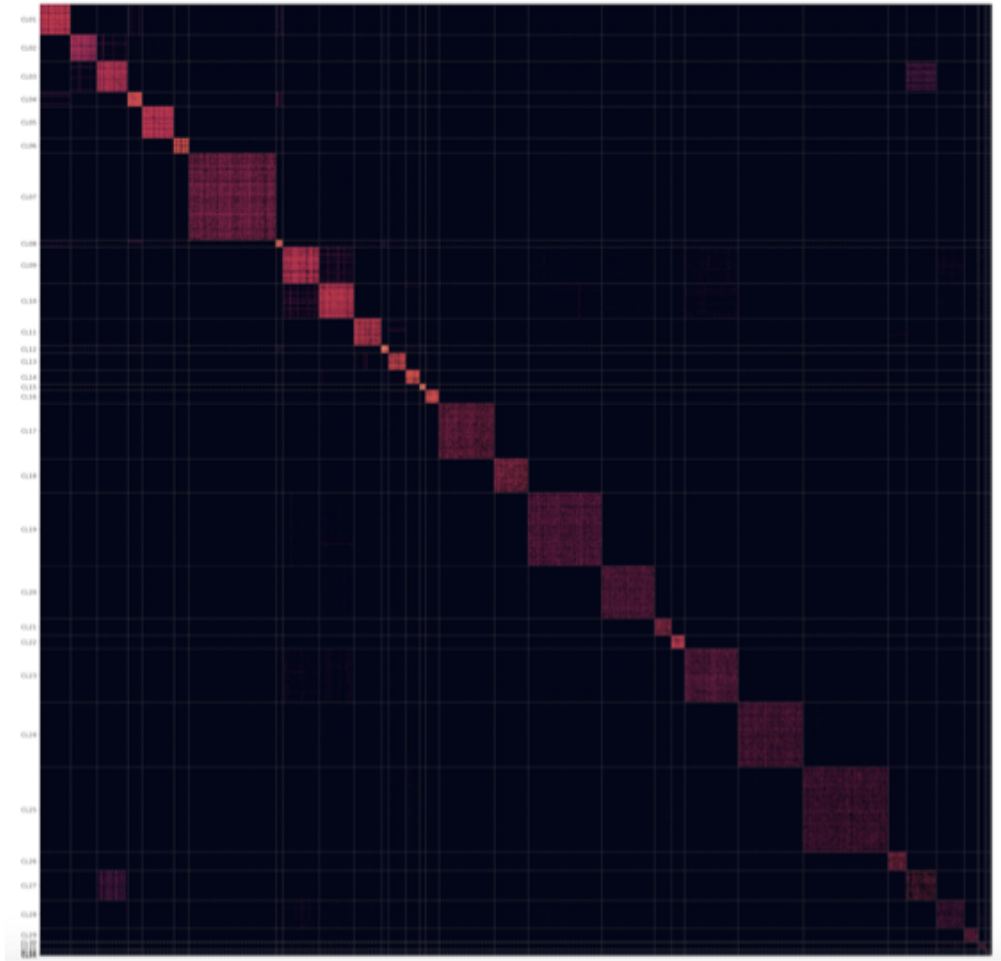
Contamination across high-quality MAGs is lower for Hi-C.

Microbiome-wide Hi-C chromosome contact maps

The Hi-C reads are mapped to WGS contigs.

For each MAG, there is a draft chromosome contact map.

How to finalize it?



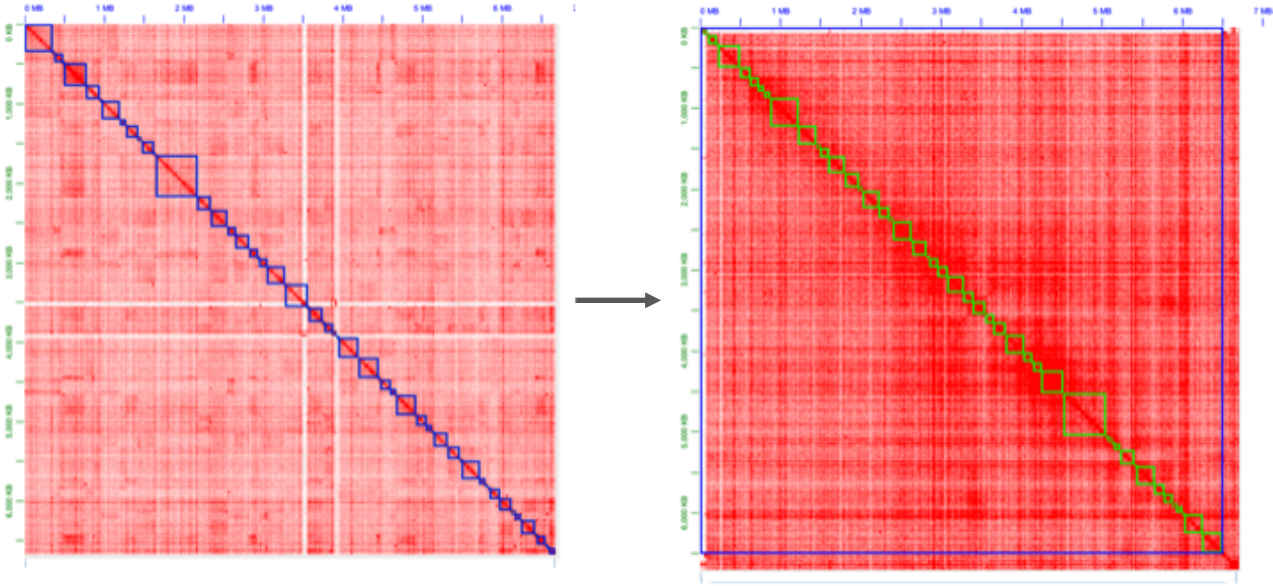
Hi-C map for all MAGs (sample 6)
(1 pixel = 1 contig)

Hi-C allows to obtain chromosome-scale scaffolds for abundant MAGs

Automatic re-ordering of the contigs based on maximization of diagonal signal.

Commonly used to improve scaffolding for single genomes of higher eukaryotes.

We applied it to microbiome-recovered maps to scaffold each MAG.



Bacteroides ovatus, sample 4
via 3D-DNA algorithm (Dudchenko et al, 2017).

Comparative genome analysis for major Hi-C MAGs of opportunist taxa

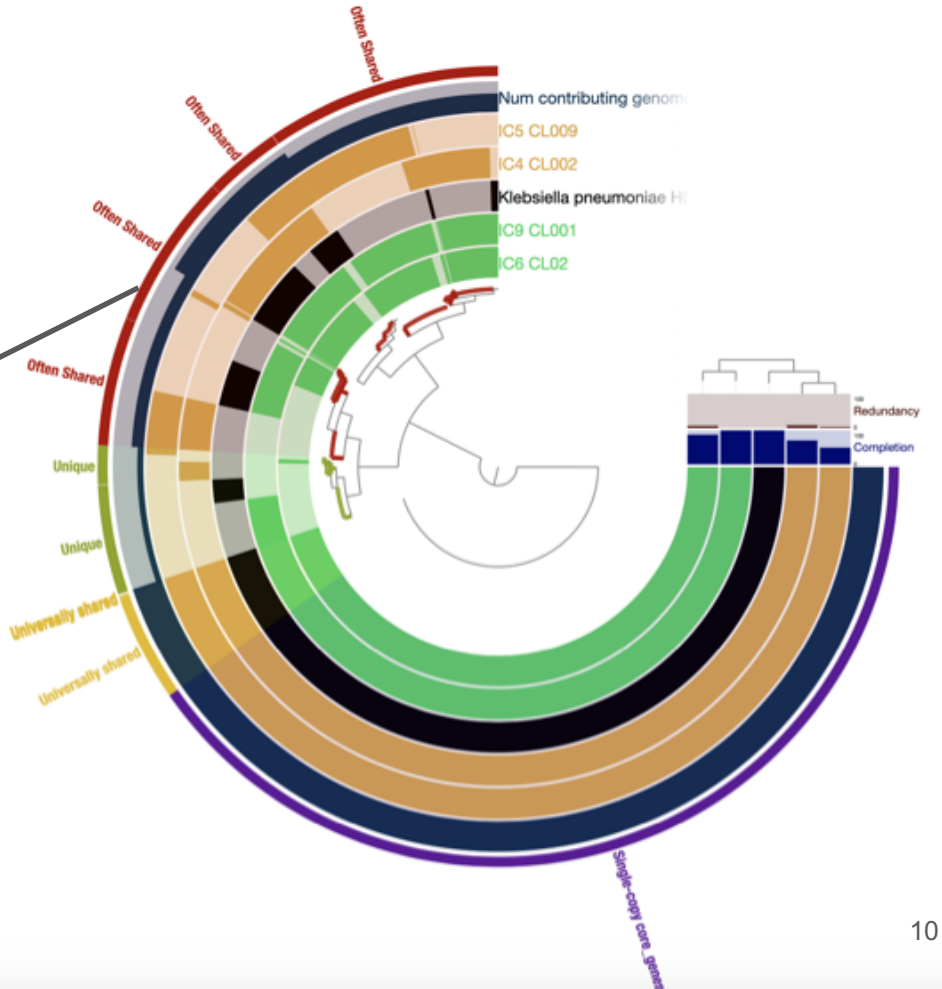
Klebsiella pneumonia (compared to external strain HS11286**)**

Within-patient genome similarity is higher than between the patients.

Patient B samples (6-9) are more similar than for A (4-5).

Interindividual differences in gene content are linked to virulence

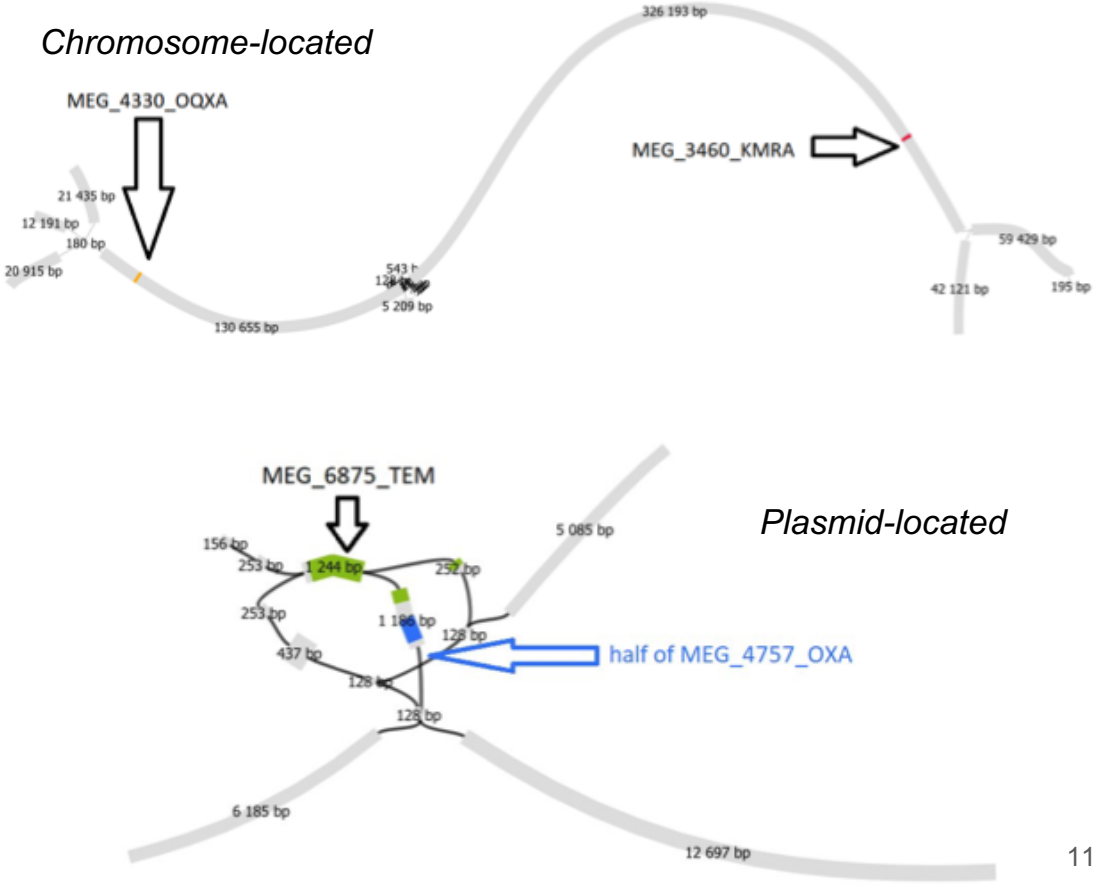
The genes unique to the patient B include the ones related to pili, fimbriae, phages and HGT.



Elucidating genomic context location of antibiotic resistance genes

Identify the environment of selected AR genes in the assembly graph.

Hi-C augmented version: in progress.



ESBL and other genes of K. pneumonia, sample 6 (using metaSPADES)

WGS shows extensive presence of plasmids

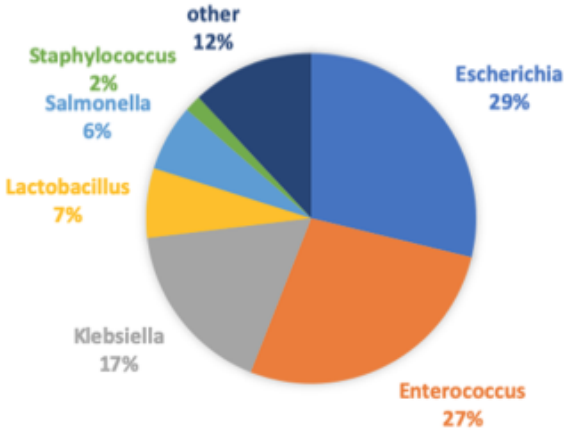
Plasmids are important HGT channel responsible for AR genes exchange.

Proteobacteria and *Enterococci* disproportionately contribute to the plasmid pool compared to the commensal taxa.

WGS allows to identify plasmid-like contigs, but not to link them to bacterial species.

Plasmids are underrepresented in WGS-MAGs.

#4



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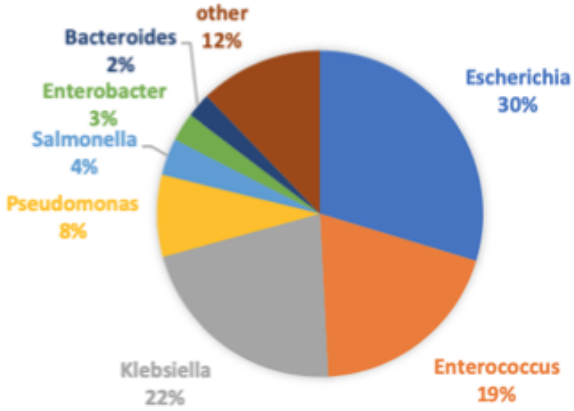
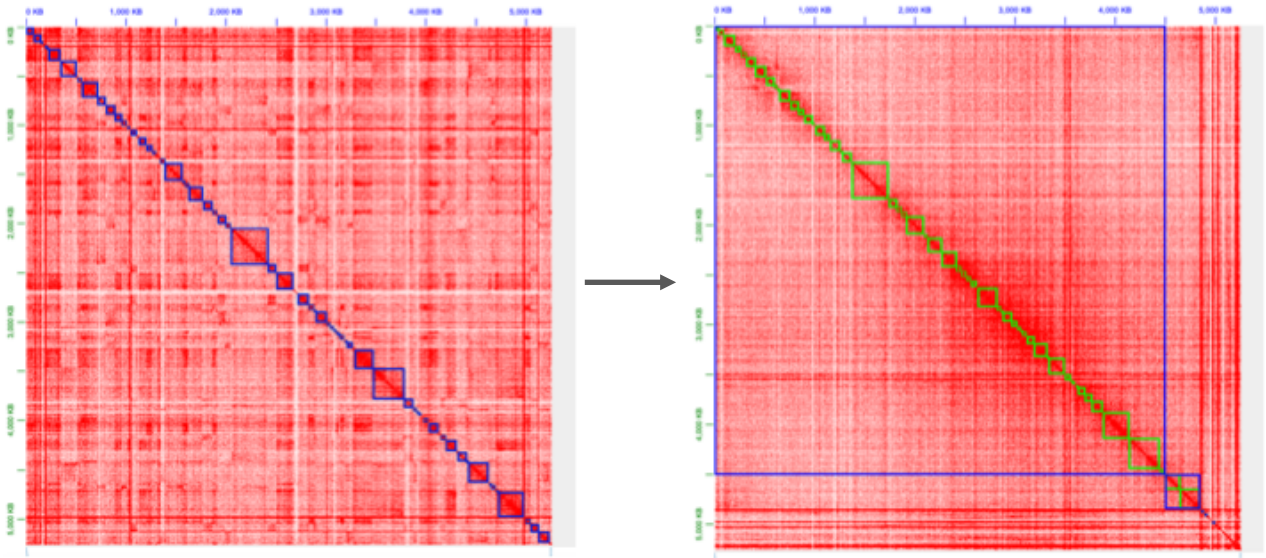


PlasFlow (# of plasmid contigs per taxon)

Hi-C data help to elucidate “host-plasmid” associations

A plasmid is linked stronger to its hosts’ chromosome by the pairs of Hi-C reads than to ones of the other microorganisms.



E. coli, sample 6

The contigs forming solid vertical lines come from plasmids

Conclusions

- Hi-C metagenomics is a promising tool for analyzing clinical microbiome samples.
- Improved Hi-C MAGs: higher completeness, lower contamination, chromosome-scale scaffolds.
- For critical care, the method allows to get insights into virulence potential and antibiotic resistance, as well as tracking mobile genetic elements dynamics.
- The findings can help optimize the treatment schemes and understand mechanisms of pathogenesis.
- The pilot pipeline is established for analyzing involvement of gut microbiome in COVID-19 pathogenesis using Hi-C metagenomics.

Thank you for your attention!

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