# Computational Molecular Biology and Bioinformatics

## Metagenomics

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# What is metagenomics?

Metagenomics is defined as the direct genetic analysis of genomes (microbial communities) contained within a site (environmental, clinical, built-in, etc.).

Metagenomics allows for a detailed study of the diversity of communities, and therefore to clarify the mechanisms of their functioning, to determine the metabolic relationships.

**Note:** In Greek, meta means "beyond or above the range of normal or physical human experience".

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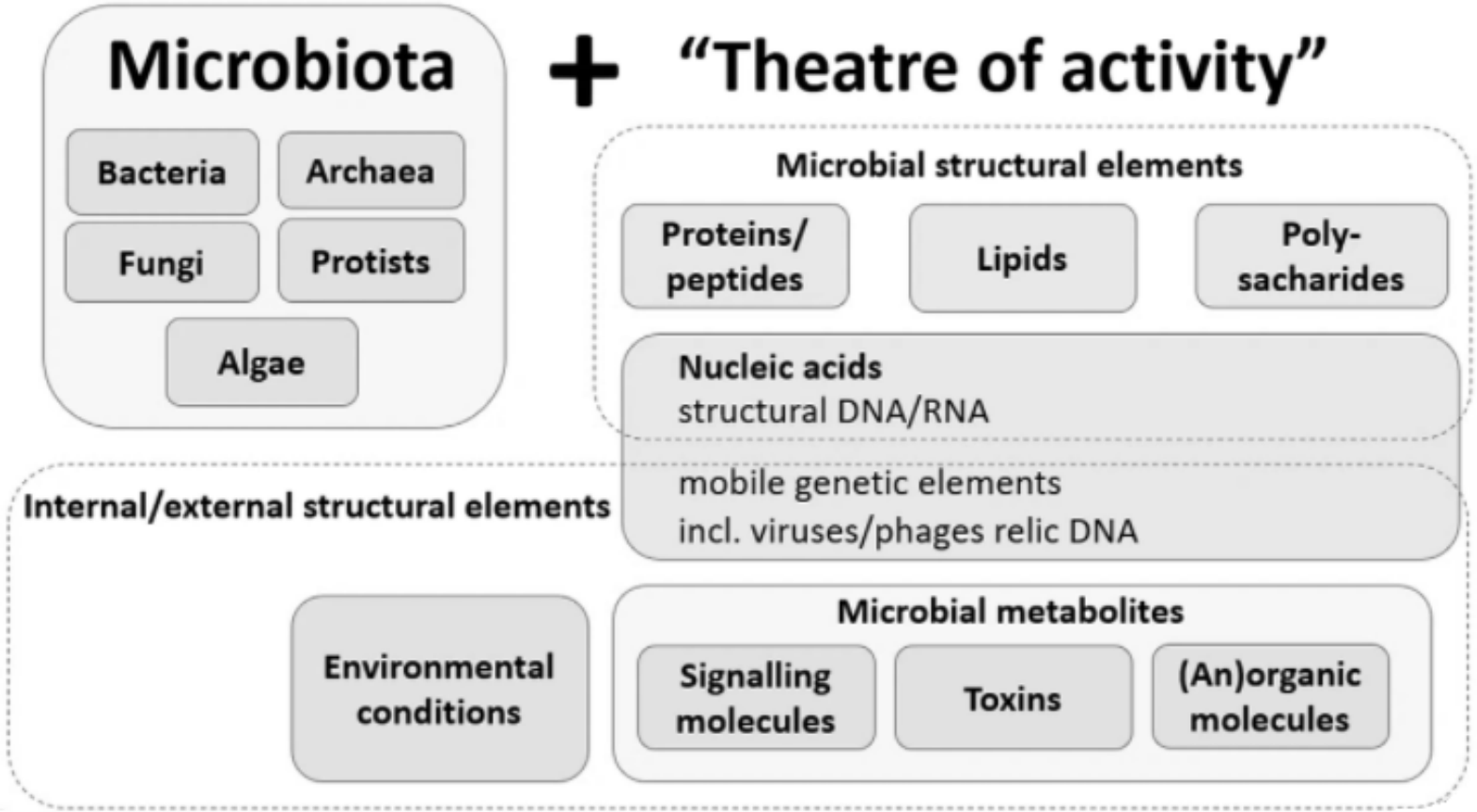
- **Symbiotic:** An organism that lives in beneficial association with the host. E.g., Bacteroides thetaiotaomicron found in human intestine.
- **Pathogenic:** An organism which is capable of causing diseases in a host. E.g., SARS-CoV-2 causing COVID-19 in human.

# What is microbiome?



# Human microbiome

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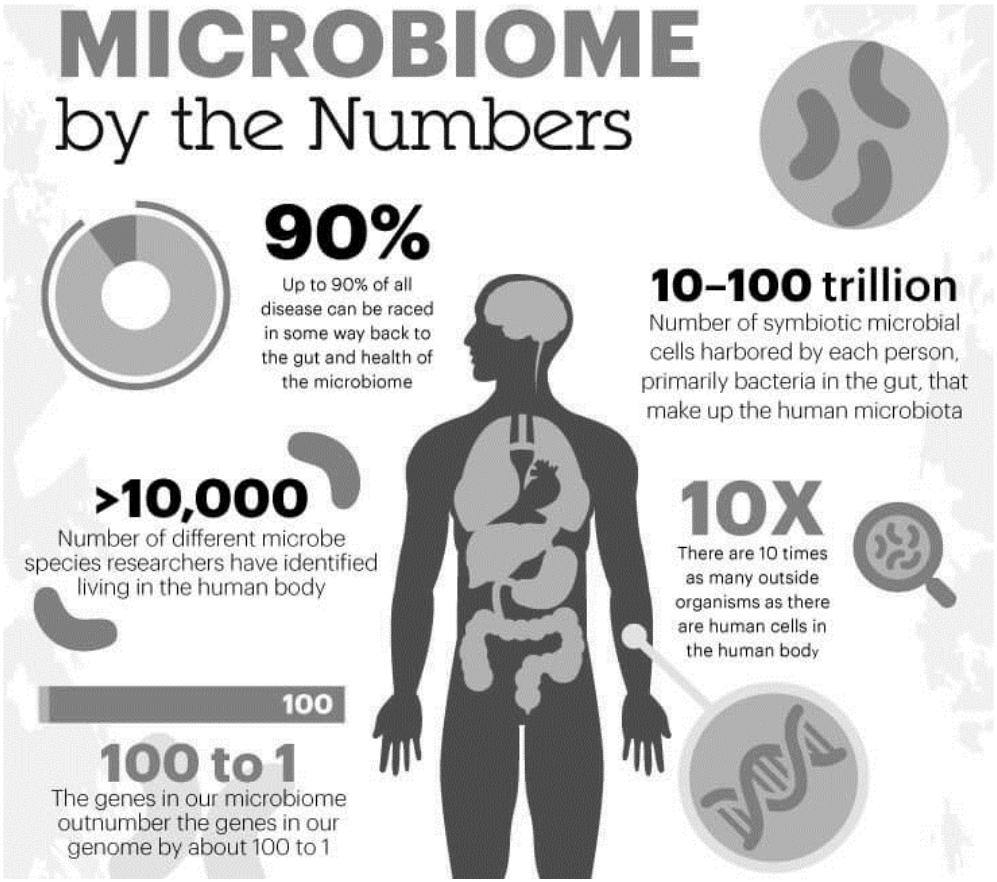
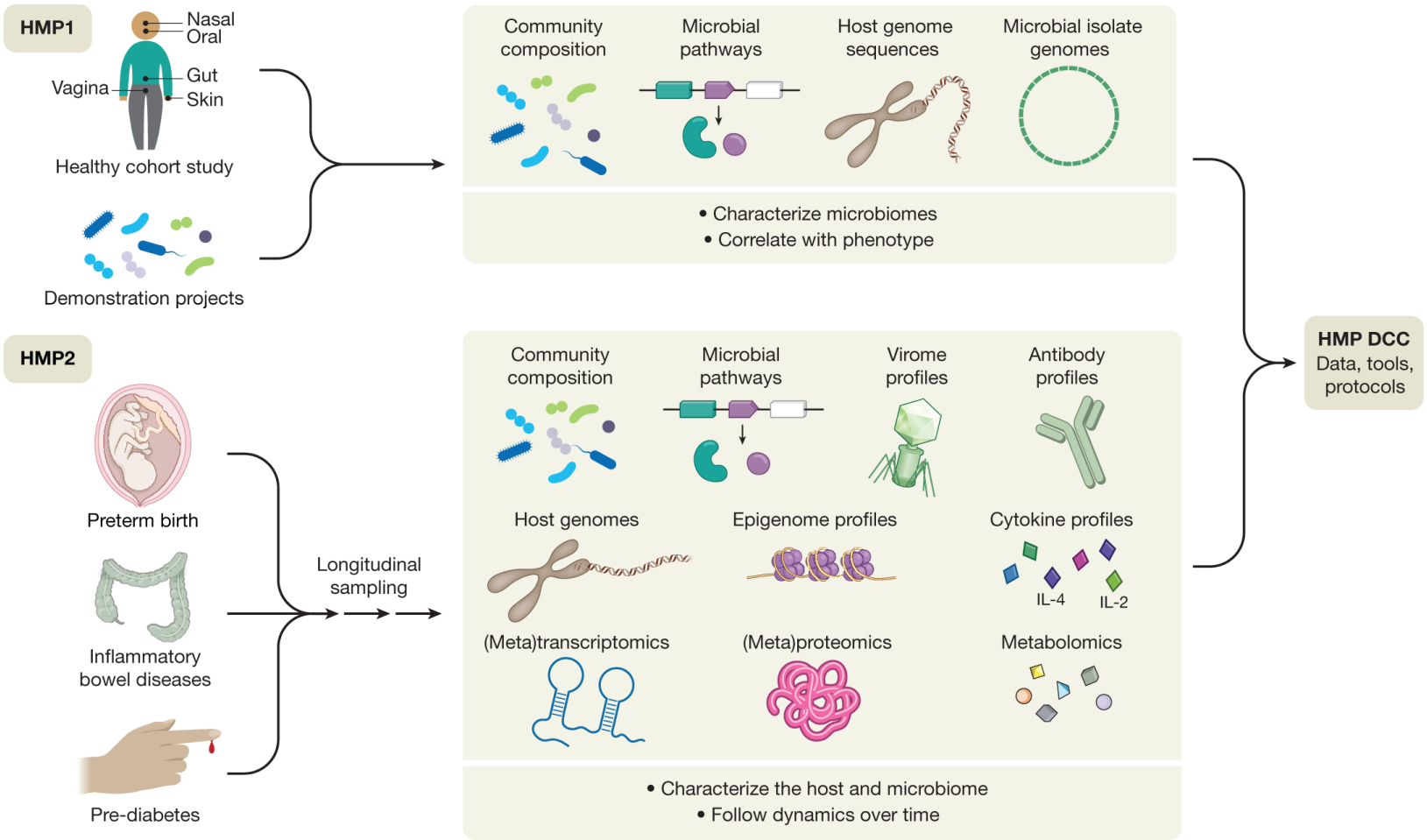


# The integrative Human Microbiome Project (iHMP)

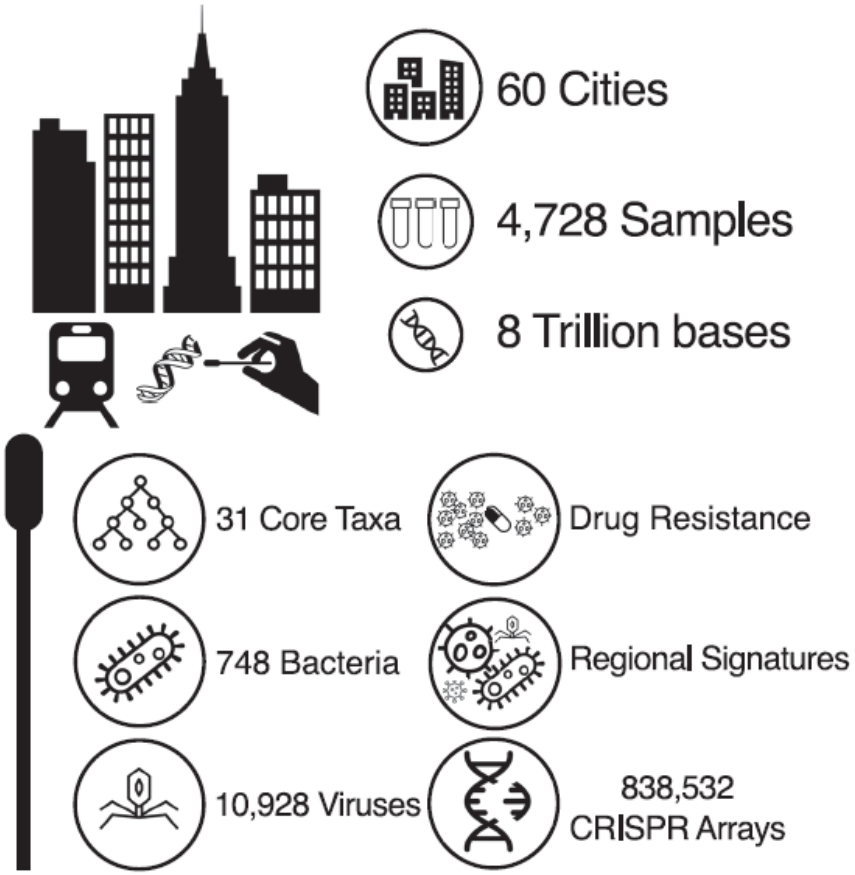



**Source:** Nature, 569:641-648, 2019.

# Microbiome of the built environment

Microbiome of the built environment comprises the communities of microorganisms that reside in human constructed environments.

# Microbiome in mass-transit systems




**Source:** Cell, 184(13):3376-3393, 2021.

# Availability of data

MetaSUB sequencing data is available at:
`https://pngb.io/metasub-2021`

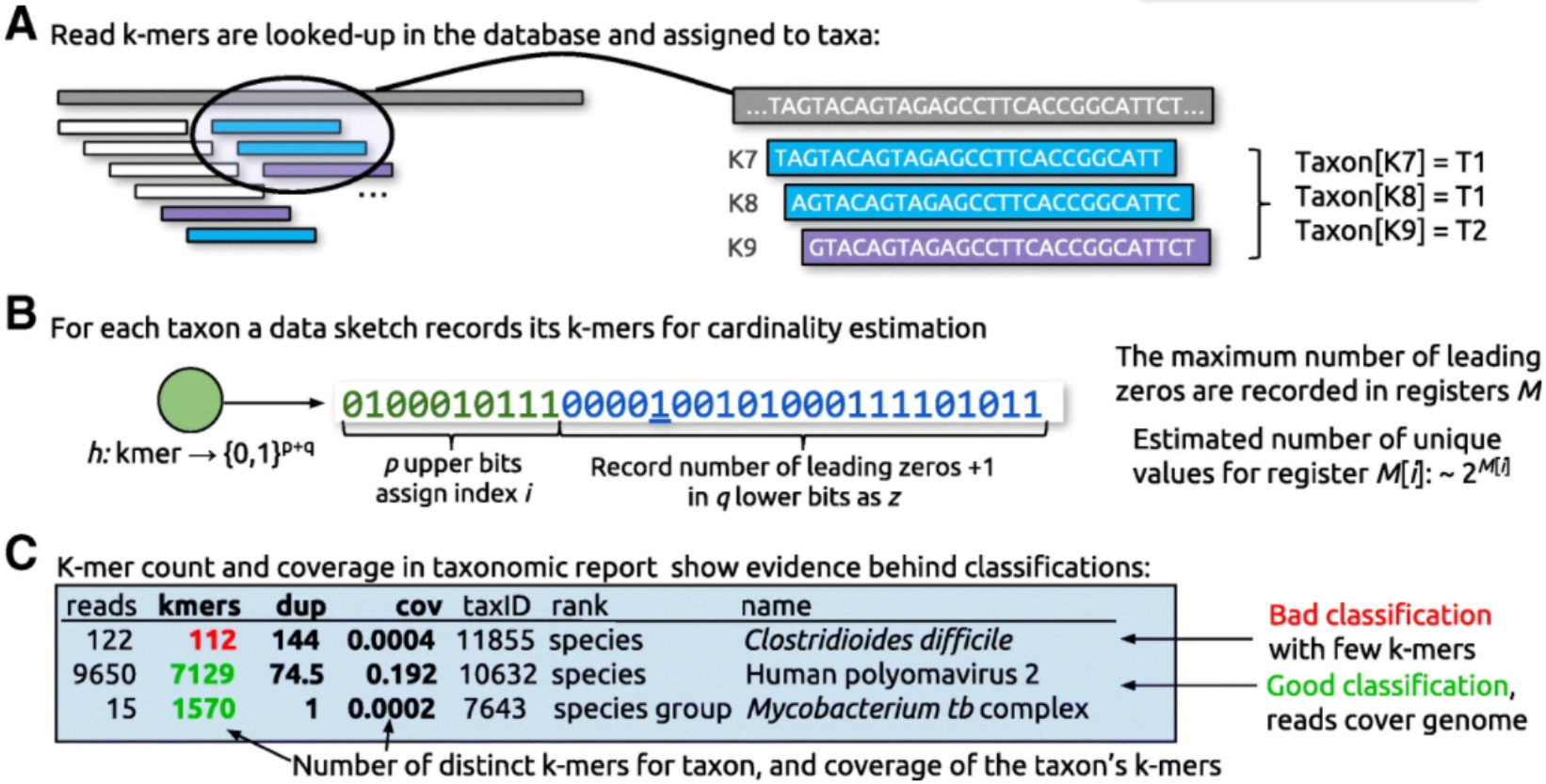
# Generating taxonomic profiles for samples

KrakenUniq (formerly KrakenHLL) is a novel metagenomics classifier that combines the fast k-mer-based classification of Kraken with an efficient algorithm for assessing the coverage of unique k-mers found in each species in a dataset.

On various test datasets, KrakenUniq gives better recall and precision than other methods and effectively classifies and distinguishes pathogens with low abundance from false positives in infectious disease samples.

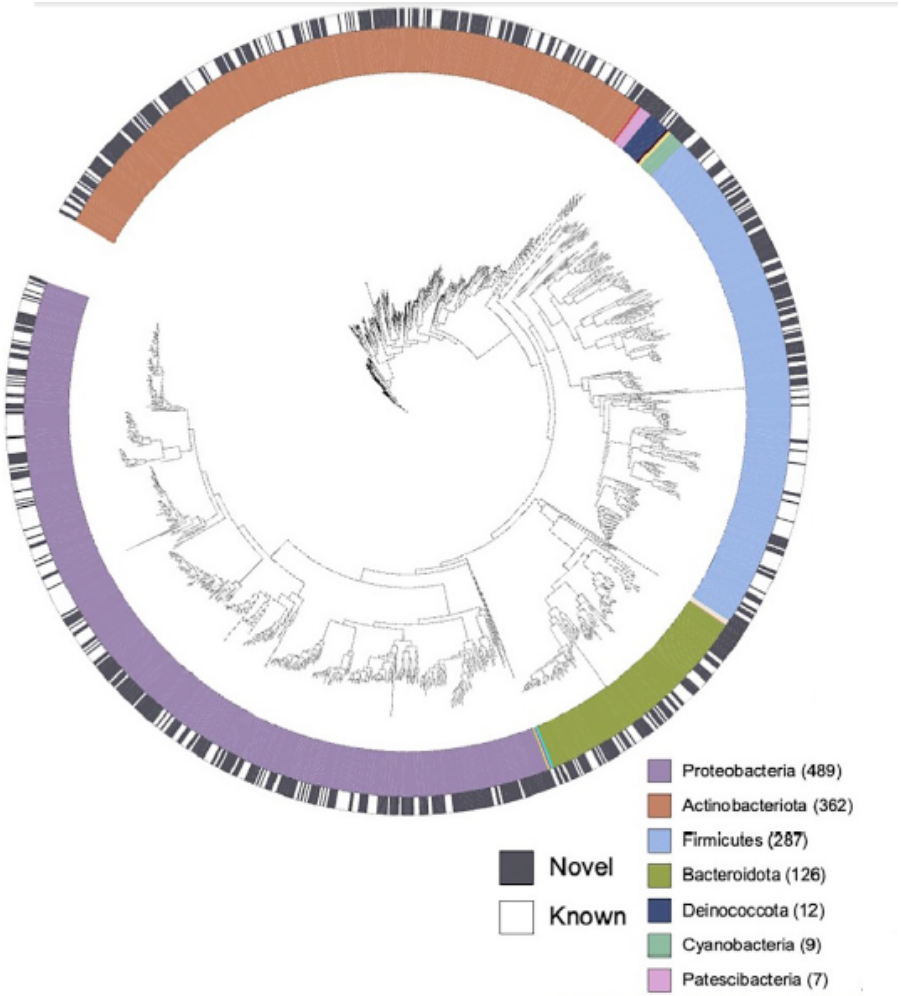
**Note:** KrakenUniq requires a huge amount of primary memory (ideally 128-512 GB). For performing more memory efficient classification, consider using Centrifuge (ideally requiring 4-12 GB).

# Overview of the KrakenUniq algorithm




**Source:** Genome Biology, 19:198, 2018.

# Taxonomic tree for metagenome-assembled genomes (MAGs)



# Dimensionality Reduction (UMAP vs t-SNE vs PCA)

| **UMAP** | **t-SNE** | **PCA** |
|---|---|---|
| 1. Uniform Manifold Approximation and Projection | 1. t-distributed Stochastic Neighbourhood Embedding | 1. Principal Component Analysis |
| 2. Unsupervised | 2. Unsupervised | 2. Unsupervised |
| 3. Non-linear | 3. Non-linear | 3. Linear |
| 4. Not deterministic | 4. Not deterministic | 4. Deterministic |
| 5. Captures the global structure of data | 5. Captures the local structure of data | 5. Captures the global structure of data |
| 6. Relatively faster | 6. Relatively slower | 6. Relatively faster |

# Analysis with UMAP

UMAP is a dimension reduction technique that can be used for visualisation of data in a lower dimension. The algorithm is founded on three assumptions about the data:

1. The data is uniformly distributed on Riemannian manifold
2. The Riemannian metric is locally constant (or can be approximated as such)
3. The manifold is locally connected

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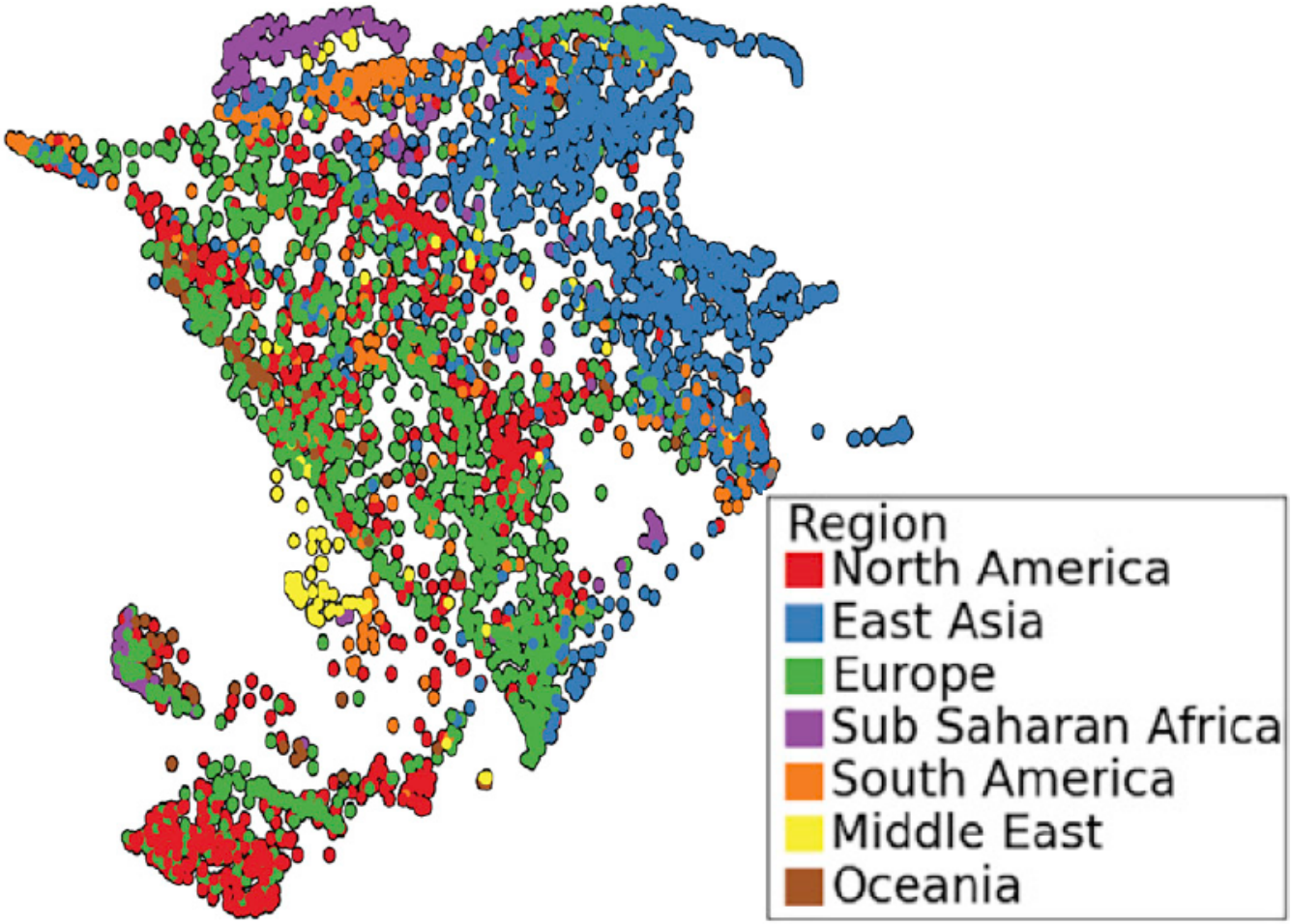
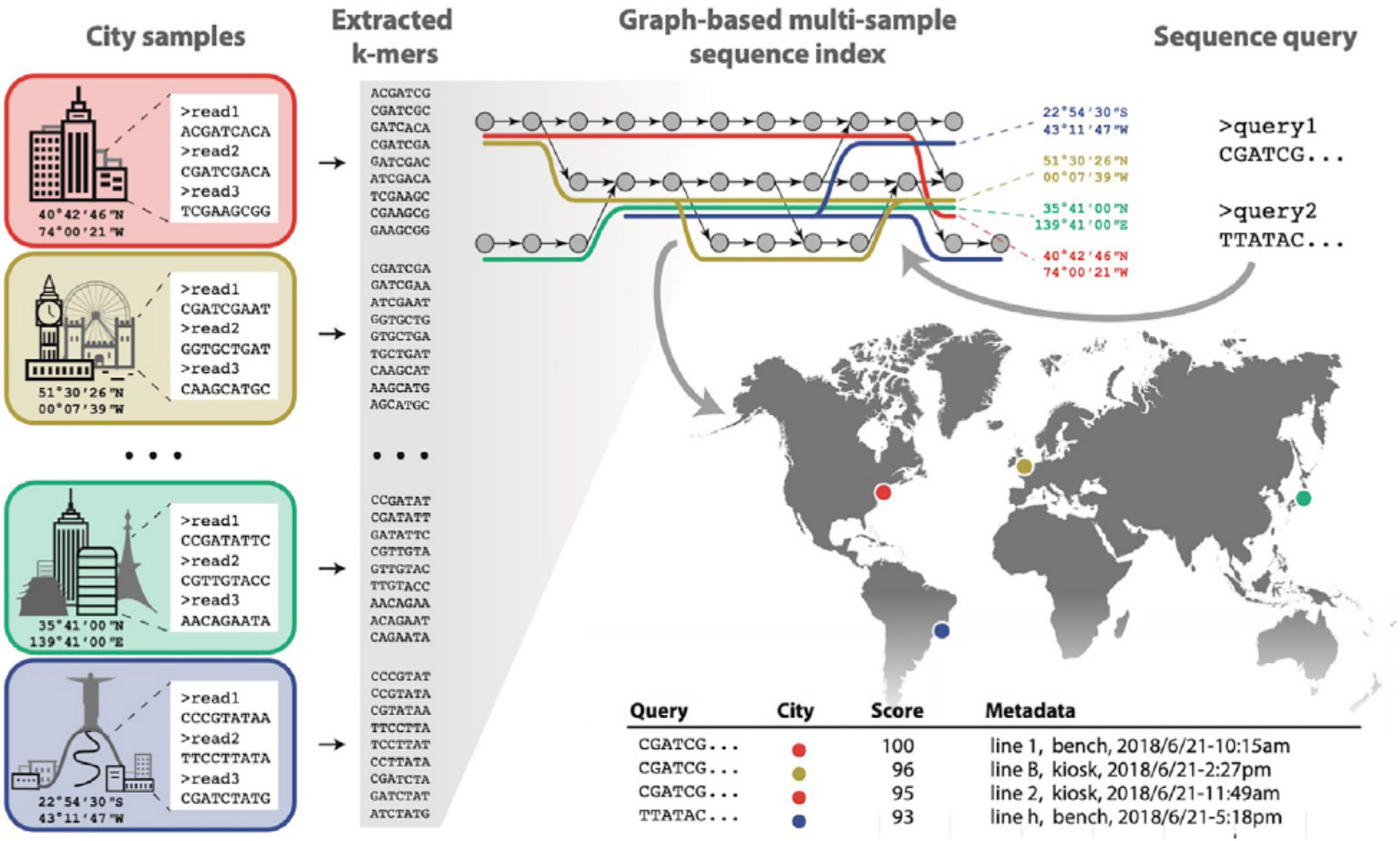
From these assumptions, it is possible to model the manifold with a fuzzy topological structure. The embedding is found by searching for a low dimensional projection of the data that has the closest possible equivalent fuzzy topological structure.

# Analysis with UMAP

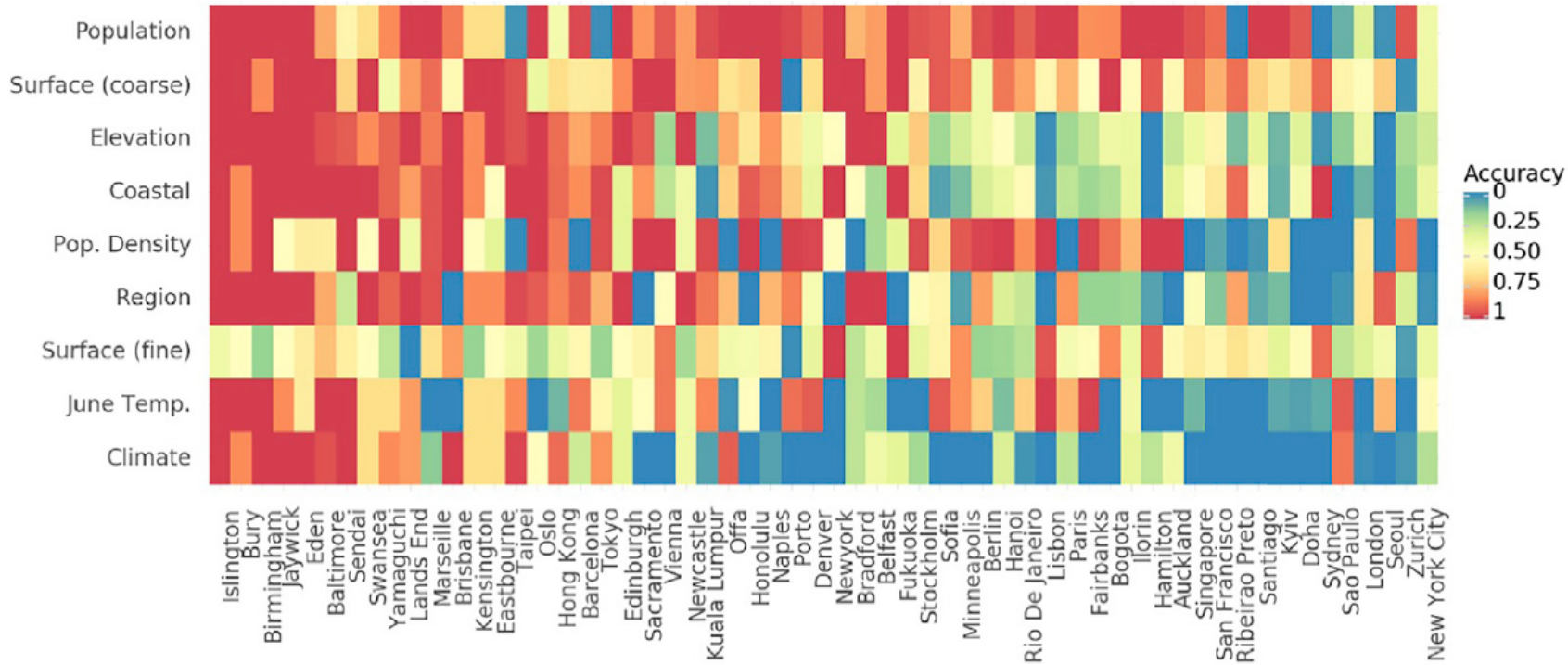


# Microbial signatures




Schematic of GeoDNA representation using indexing on graphs

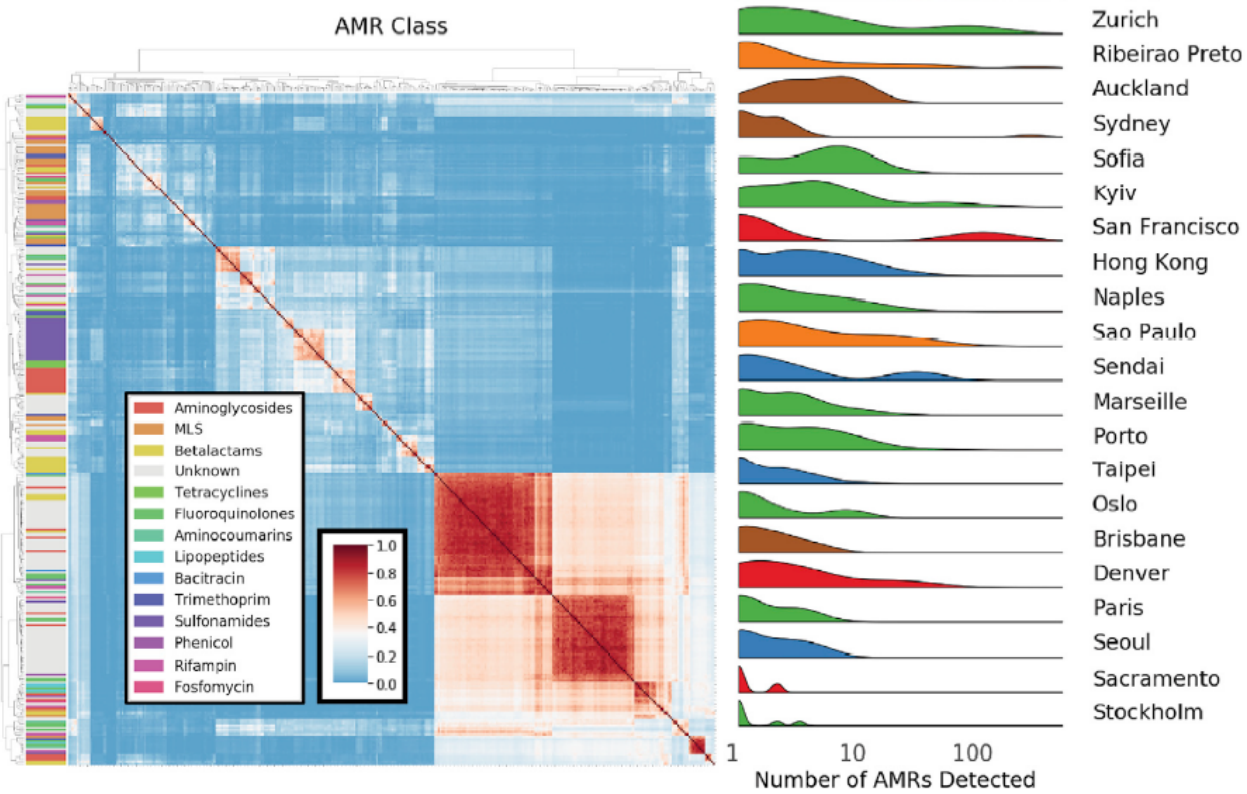
# Prediction of features




Prediction accuracy of a random forest model for a given feature

# Antimicrobial resistance

Using the MegaRES ontology and alignment software, one can map reads to known antibiotic resistance genes.




Co-occurrence of AMR genes (left), AMR genes by city (right)

# Software and algorithms

| Tool | Availability |
|---|---|
| AdapterRemoval v2.17 | https://github.com/mikkelschubert/adapterremoval |
| Bowtie2 v2.3.0 | https://sourceforge.net/projects/bowtie-bio/files/bowtie2/2.3.0 |
| BLASTn | https://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST |
| KrakenUniq v0.3.2 | https://github.com/fbreitwieser/krakenuniq |
| Centifuge | https://github.com/infphilo/centrifuge |
| MASH v2.1.1 | https://github.com/marbl/Mash |
| HUMAnN2 | https://pypi.org/project/humann2 |
| DIAMOND v0.8.36 | https://github.com/bbuchfink/diamond |
| metaSPAdes v3.8.1 | https://github.com/ablab/spades/releases/tag/v3.8.1 |
| MegaRes v1.0.1 | https://megares.meglab.org/download/index.php |
| MetaBAT2 v2.12.1 | https://anaconda.org/ursky/metabat2 |
| CheckM v1.0.13 | https://github.com/Ecogenomics/CheckM |
| dnadiff v1.3 | https://github.com/mummer4/mummer |
| GTDB-Tk v1.0.2 | https://github.com/jianshu93/GTDB_Tk |
| FastTree v2.1.10 | https://anaconda.org/bioconda/fasttree |
| iTOL v5.5 | https://itol.embl.de |

# Hands-on

1. Read the following paper and access its data.
   i) D. Danko et al., A global metagenomic map of urban microbiomes and antimicrobial resistance. Cell, 184(13), pp.3376-3393, 2021.
   Link: `https://www.sciencedirect.com/science/article/pii/S0092867421005857`.