EBAii Assemblage & Annotation

Part 2: construction and analysis of procaryotic genomic dataset

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2. Construction and analysis of procaryotic genomic dataset

Outline

> 2.1 Downloading a dataset of public genomes

> 2.2 Analyzing the genome dataset

> 2.3 Comparing and dereplicating the dataset

Many slides from the "***Bioinformatique par la pratique***" migale training cycle "Comparison of microbial genomes" module

https://migale.inrae.fr/trainings

And thanks to Guillaume Gautreau for his help

Hélène Chiapello
Training

Valentin Loux
Technical coordinator

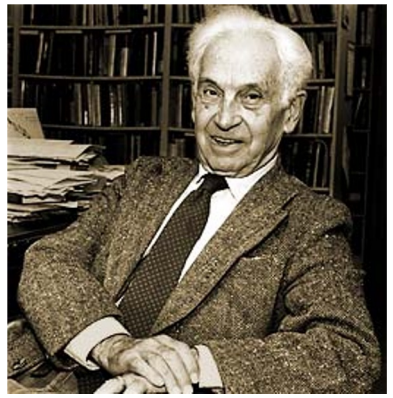
2.2 Analyzing a genome dataset

Why?

- Frequent problems in genome analysis and comparison
  - Heterogenous quality of sequencing and assembly
  - Presence of huge number or public genomes OR absence of any close genomes of the same species in public databases
  - Difficulties regarding microbial taxonomy (classification) and nomenclature (naming of genus, species and strain naming) for many non-model organisms
- Outline
  - 2.2.1 Introduction
  - 2.2.2 Dataset diversity analysis
  - 2.2.3 Dataset quality analysis

2.2.1 Introduction

- What is a species?



Ernst Mayr (1942) :

“Species are groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups”

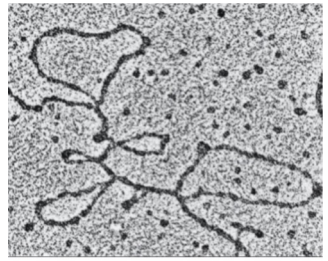
⇨ ***Not relevant for bacteria***

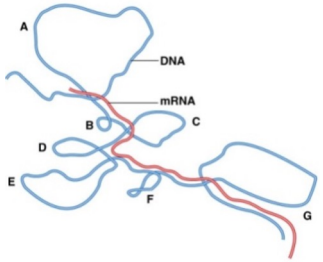
What is a bacterial species?

No consensual definition for procaryotes

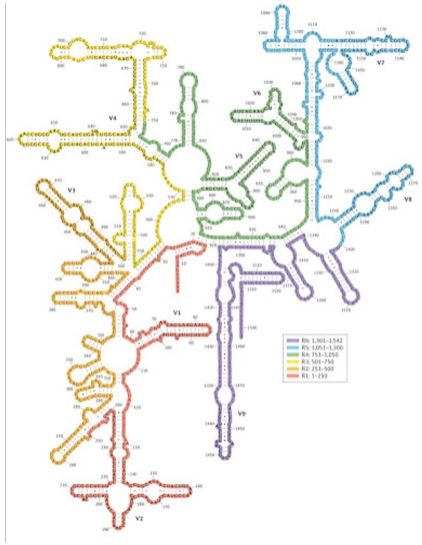
- No universal criteria
- Several approaches used to classify bacterial
  - Phenotypes and morphological criteria
  - DNA-DNA hybridization
- Universal markers
  - 16S rRNA
  - MLST (Multi Locus Sequence Typing)
- Genomic-based taxonomy are now becoming a gold-standard

% ADN-ADN hybridation	>70%





% rRNA 16S identity	>98,7%

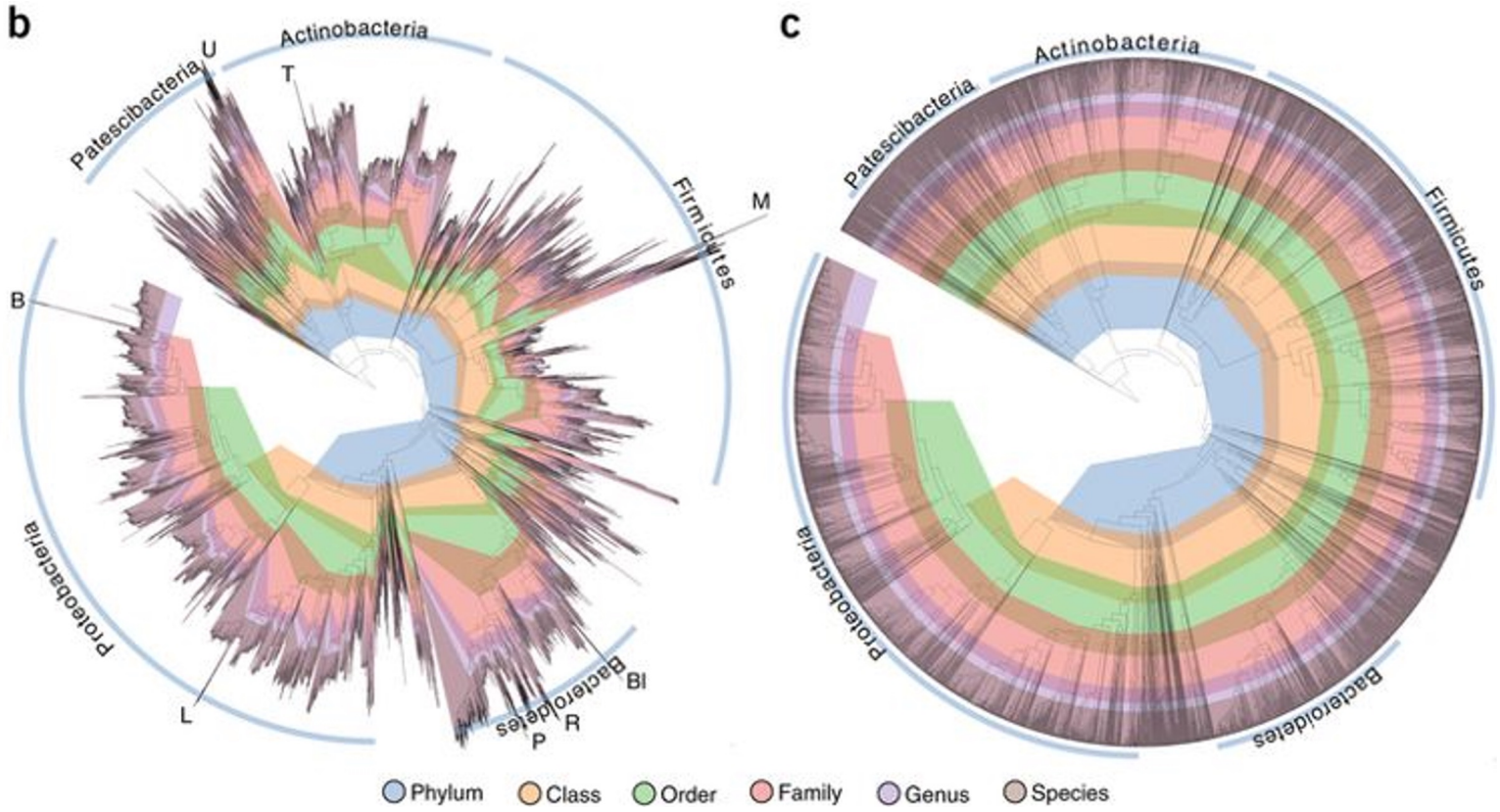


Example: the Genome-based taxonomy for prokaryotic genomes

- Objective: a standardized microbial taxonomy based on genome phylogeny
- Taxonomy inferred from concatenated single copy marker proteins

Parks et al. 2018, 2021

https://gtdb.ecogenomic.org/



2.2.2 Evaluating genome diversity in a dataset

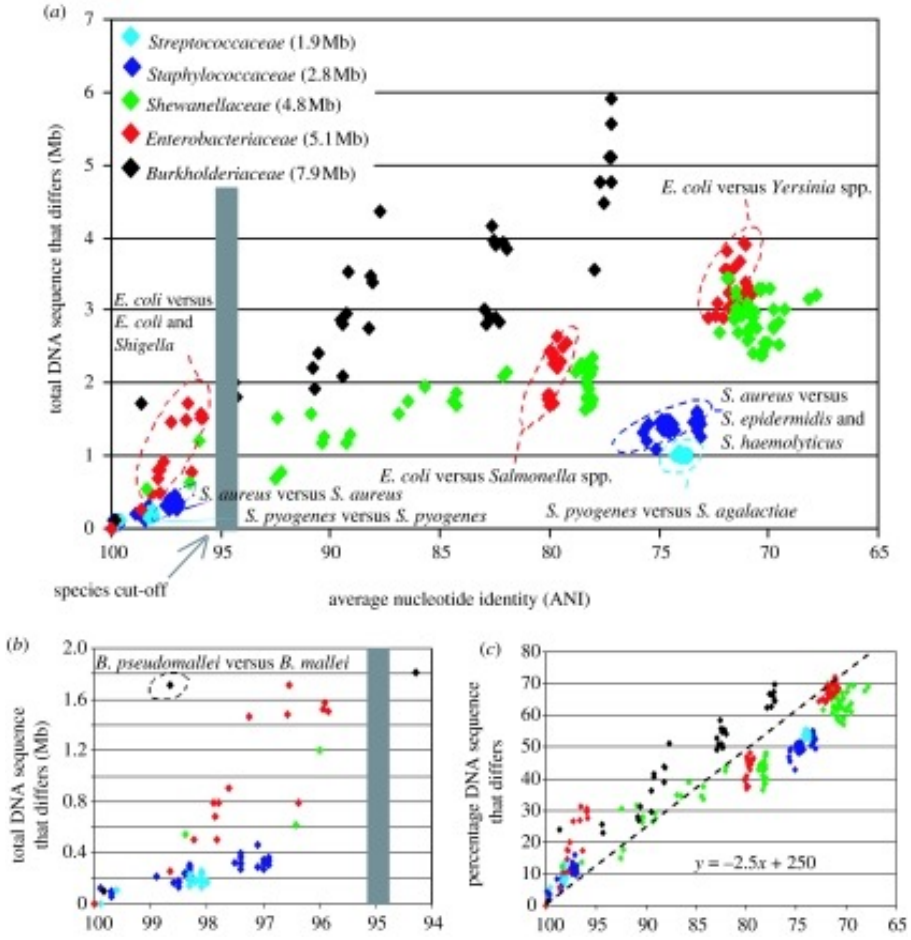
- Why ?
  - Identify outlier genomes
  - Identify groups of (very) similar genomes and de-replicate datasets
  - Estimate genome similarity in a dataset and design an adapted comparative strategy

How ?

- Alignment based approaches (ANI)
- k-mer based approaches (MASH)

Average Nucleotide Identity (ANI)

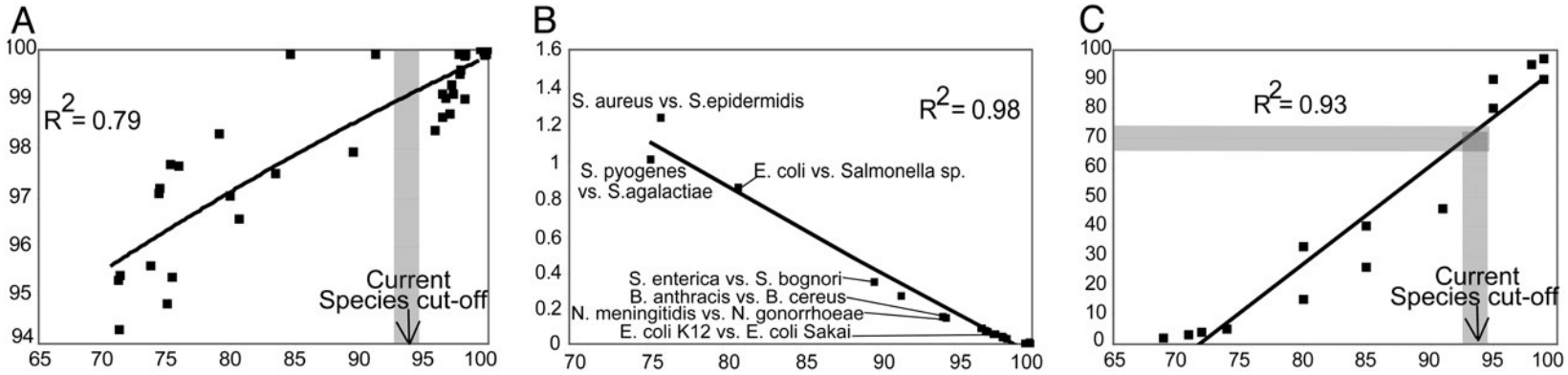
- Meet the need for a robust measure of genomic relatedness and a systematic and scalable species assignation technique
- Mean identity percent of aligned regions of a pair of genomes
- Rely on pairwise alignments from
  - aligned core genes
  - genomic alignments
- Can easily be used to build phylogenetics tree using distance methods
- Is implemented in several bioinformatics tools: ANIn (nucmer based, Richter 2009) gANI (coding regions, Varghese 2015),…



Genetic diversity within five important bacterial groups. Konstantidinis et al. 2006. The bacterial species definition in the genomic era
DOI: 10.1098/rstb.2006.1920

Average Nucleotide Identity (ANI)

- ANI strongly correlates (R = 0.79 for logarithmic correlation) with the 16S rRNA gene sequence identity and can resolve areas where the 16S rRNA gene is inadequate (intra-species level)
- The average rate of synonymous substitutions shows a tight correspondence to ANI, suggesting that ANI may also be a useful descriptor of the evolutionary distance
- ANI shows a strong linear correlation to DNA–DNA reassociation values, and the 70% DNA–DNA reassociation standard corresponds to ≈93–94% ANI i.e. strains that show >94% ANI should belong to the same species

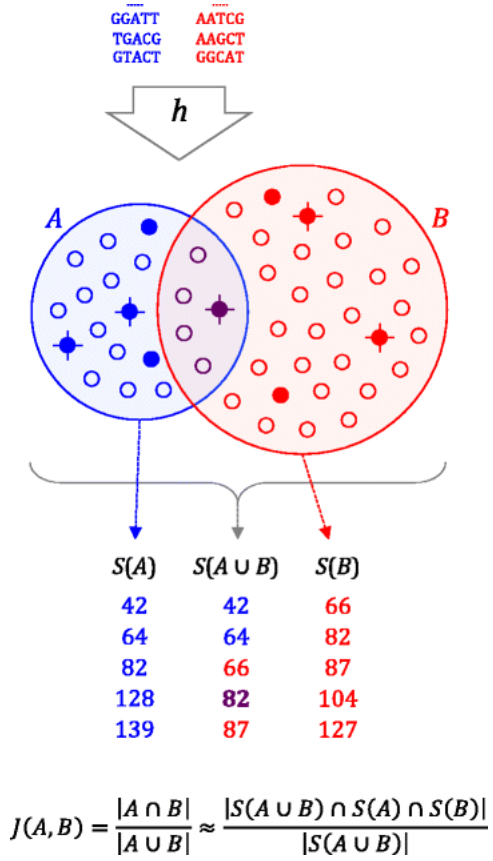


ANI vs 16 rRNA %id | ANI vs average syn. Mutation rate | ANI vs DNA-DNA reassociation value

Konstantidinis et al. 2005. Genomic insights that advance the species definition for prokaryotes https://doi.org/10.1073/pnas.0409727102

MASH: fast (meta)genome distance estimation using MinHash

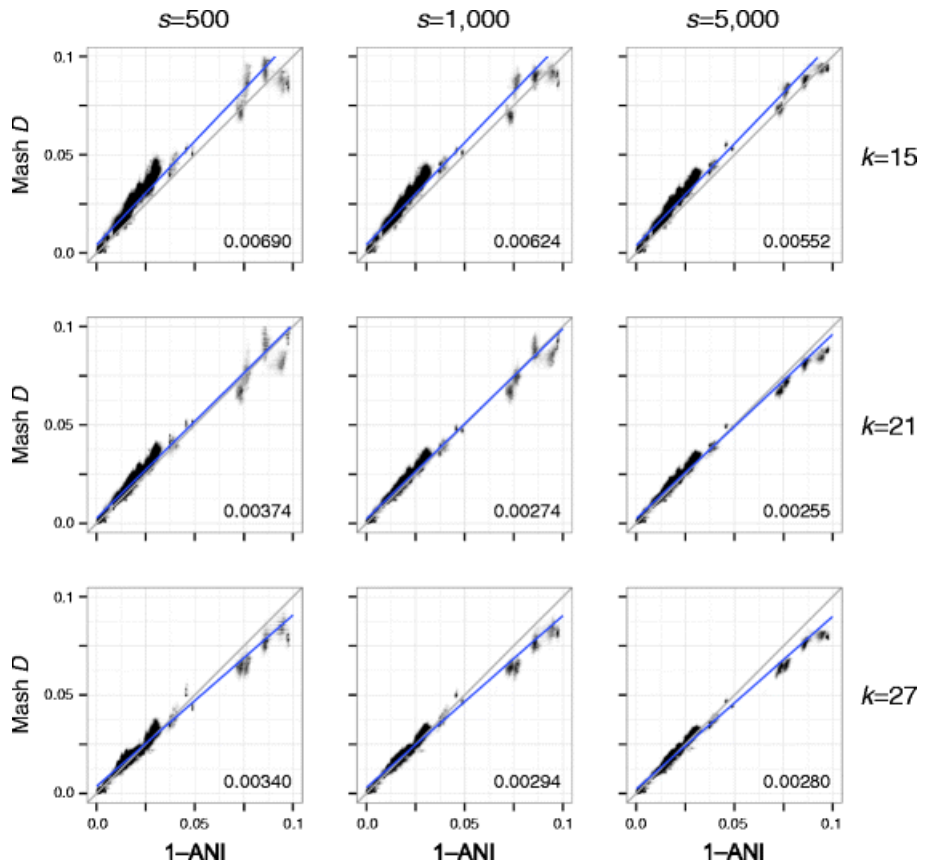
- Mash allows to compute a pairwise mutation distance without alignment using k-mer counts
- Mash provides two basic functions for sequence comparisons:
  - **sketch**: converts a sequence or collection of sequences into a MinHash sketch
  - **dist**: compares two sketches and returns an estimate of the Jaccard index (i.e. the fraction of shared k- mers), a P value, and the Mash distance



Ondov, B.D., Treangen, T.J., Melsted, P. et al. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol 17, 132 (2016). https://doi.org/10.1186/s13059-016-0997-x

MASH distances correlate well with ANI

- Dataset: 500 complete E. coli genomes
  - Each plot column shows a different sketch size
  - Each plot row a different k-mer size k.
  - Gray lines: model relationship D = 1–ANI
- Increasing the sketch size improves the accuracy of the MASH distance, especially for more divergent sequences.
- Limit on how well the MASH distance can approximate ANI, especially for more divergent genomes (e.g. ANI considers only the core genome)



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Back to procaryote taxonomy

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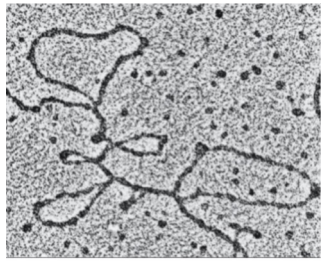

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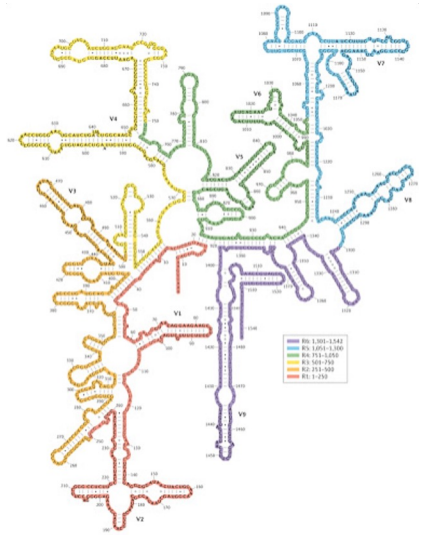
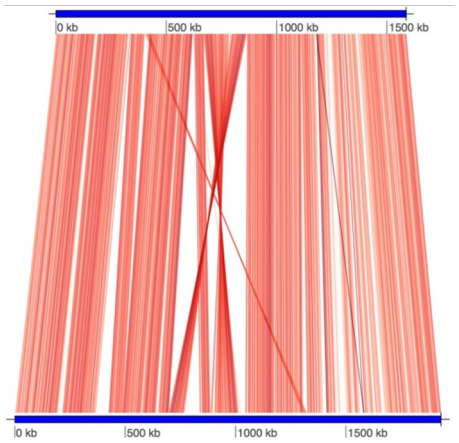
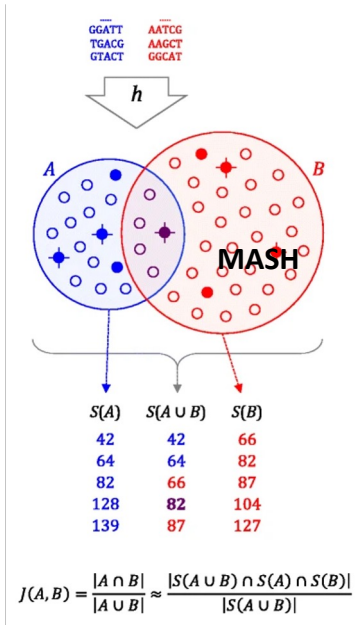


% genome identity (ANI)	>94%



K-mer distance	<0,06



2.2.3 Quality analysis

- Already presented: evaluate Quality using the 3Cs

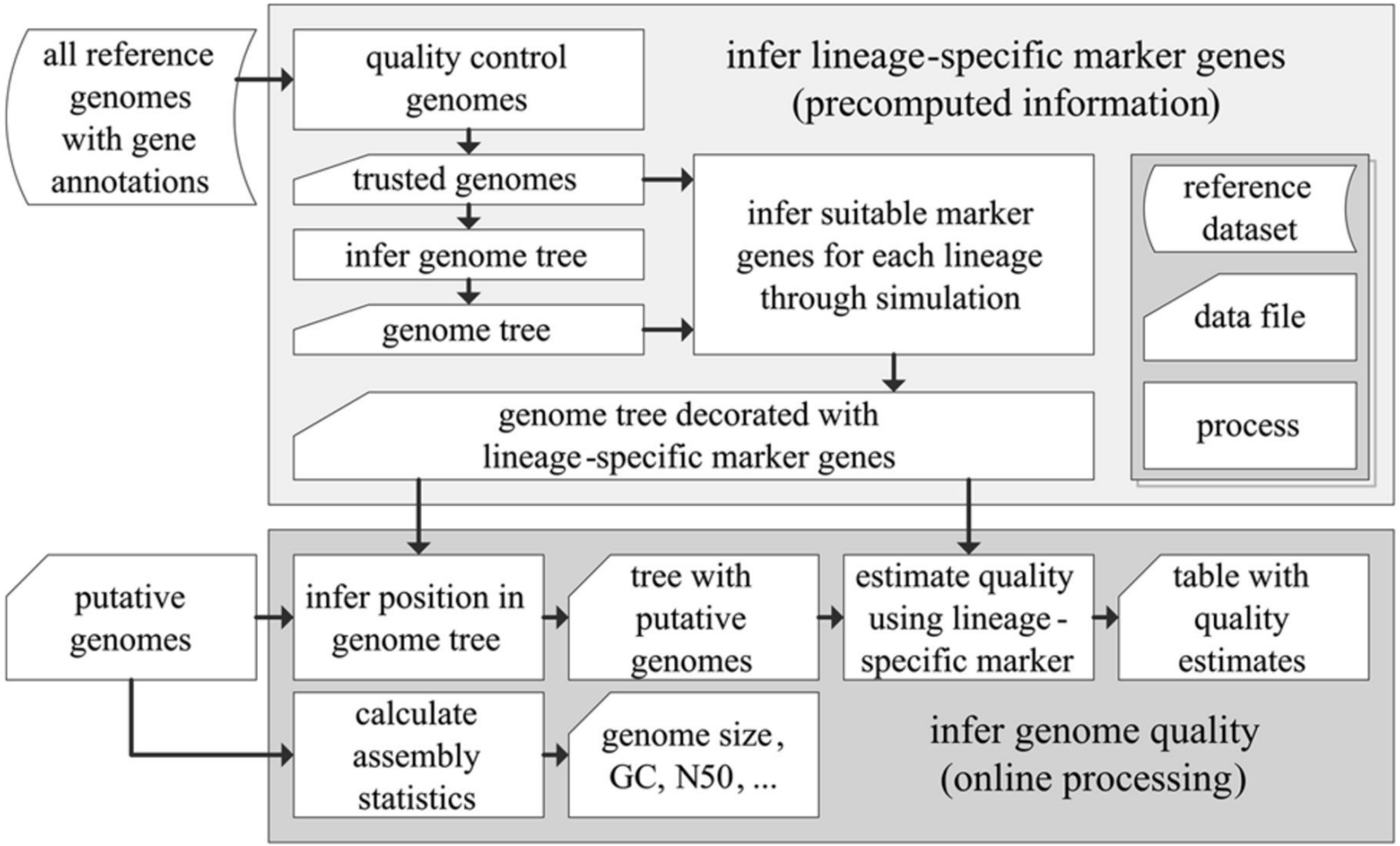
1. **Contiguity**. Produce the longest possible contigs.
2. **Correctness**. Assemble contigs with few/no errors.
3. **Completeness**. Cover the entire original sequence and minimize missing regions

- An additional key point for microbes: evaluate **Contamination**
  - From genomic fragments of divergent taxa
  - From genomic fragments of multiple strains (i.e. strain **heterogeneity**)

CheckM

- a set of tools for assessing the quality of genomes recovered from isolates, single cells, or metagenomes
- provides robust estimates of genome **completeness** and **contamination**
  - use collocated sets of genes that are ubiquitous and single-copy within a phylogenetic lineage
  - propose a fixed vocabulary for defining genome quality based on estimates of completeness and contamination
- Evaluate by simulations the accuracy of quality estimates

CheckM consists of a workflow for precomputing lineage-specific marker genes for each branch within a reference genome tree (top box) and an online workflow for inferring the quality of putative genomes (bottom box).



CheckM relies on several other tools and data

- *prodigal* to predict genes
- A reference genome tree based on 43 phylogenetically informative marker genes and 5656 trusted reference genomes
  - Marker genes are identified in assemblies using HMMER
  - The resulting genes are used to placed the genome into the tree using *pplacer*
- Lineage-specific marker sets determined for all nodes within the reference genome tree by identifying single-copy genes present in ≥97% of all descendant genomes.

CheckM report

Provides classic quality metrics and plots, including:

- Results of binning

  >Marker lineage, #genomes, #markers, #marker sets

- CheckM metrics

  > Completeness, Contamination, Strain heterogeneity

- Classical Quality metrics

> #ambiguous bases, #scaffolds, #contigs, N50 (scaffolds), N50 (contigs), Mean scaffold length (bp),Mean contig length (bp), Longest scaffold (bp),Longest contig (bp), GC, GC std (scaffolds > 1kbp)

CheckM report – binning part

Marker lineage: indicates the taxonomic rank of the lineage-specific marker set used to estimated genome completeness, contamination, and strain heterogeneity.

#genomes: number of reference genomes used to infer the lineage-specific marker set

#markers: number of marker genes within the inferred lineage-specific marker set

#marker sets: number of co-located marker sets within the inferred lineage-specific marker set

0-5+: number of times each marker gene is identified

CheckM report

- Completeness: estimated completeness of genome as determined from the presence/absence of marker genes and the expected colocalization of these genes
- Contamination: estimated contamination of genome as determined by the presence of multi-copy marker
- Strain heterogeneity: % determined from the number of multi-copy marker pairs which exceed a specified **amino acid identity threshold** (default = 90%).
  - High strain heterogeneity suggests the majority of reported contamination is from one or more closely related organisms (i.e. potentially the same species),
  - Low strain heterogeneity suggests the majority of contamination is from more phylogenetically diverse sources

CheckM: proposed genome quality classification scheme

- ***Finished genomes***: genomes assembled into a single contiguous sequence containing no gaps or ambiguities and where extensive efforts have been made to identify errors
- ***Noncontiguous finished:*** *g*enomes assembled into multiple sequences as a result of repetitive regions, but otherwise of a finished quality
- ***Draft genomes:*** all other genomes

Table 3. Controlled vocabulary of draft genome quality based on estimated genome completeness and contamination

Completeness	Classification	Contamination	Classification
≥90%	Near	≤5%	Low*
≥70% to 90%	Substantial	5% to ≤10%	Medium
≥50% to 70%	Moderate	10% to ≤15%	High
<50%	Partial	>15%	Very high

(*) Genomes estimated to have 0% contamination can be designated as having "no detectable contamination".

Donovan H. Parks et al. Genome Res. 2015;25:1043-1055

CheckM result interpretation limits

- CheckM is dedicated to eubacterial and archeal genomes
  - Eukaryotic or phage genomes will be reported as highly incomplete
  - The quality of plasmids must also be assessed independently of CheckM
- The novelty of a genome will also influence the accuracy of CheckM estimates
  - Estimates for bacterial and archaeal genomes from deep basal lineages with few reference genomes are generally based on domain-level marker sets
  - Quality estimates may be not reliable for genomes of novel lineages
  - Gene loss or duplication may be an issue

Conclusion : use CheckM as a tool to detect outliers and further investigate!

Questions ?