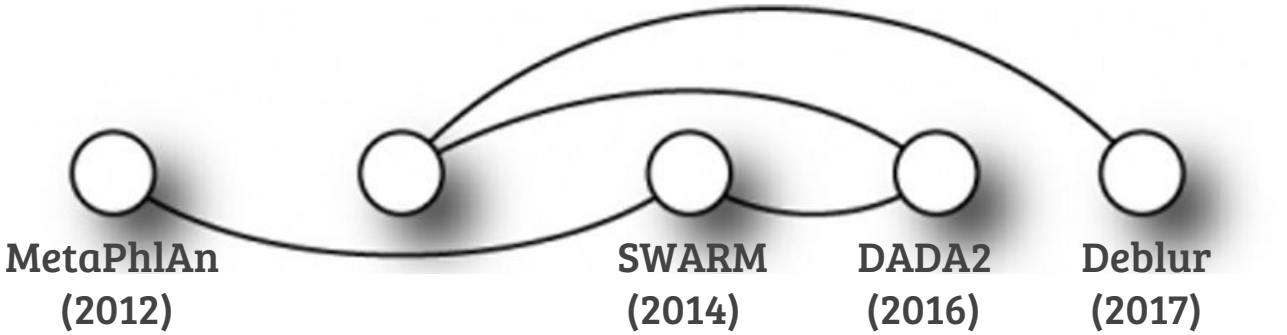
# MetaPhlAn

Metagenomic Phylogenetic Analysis

bioBakery

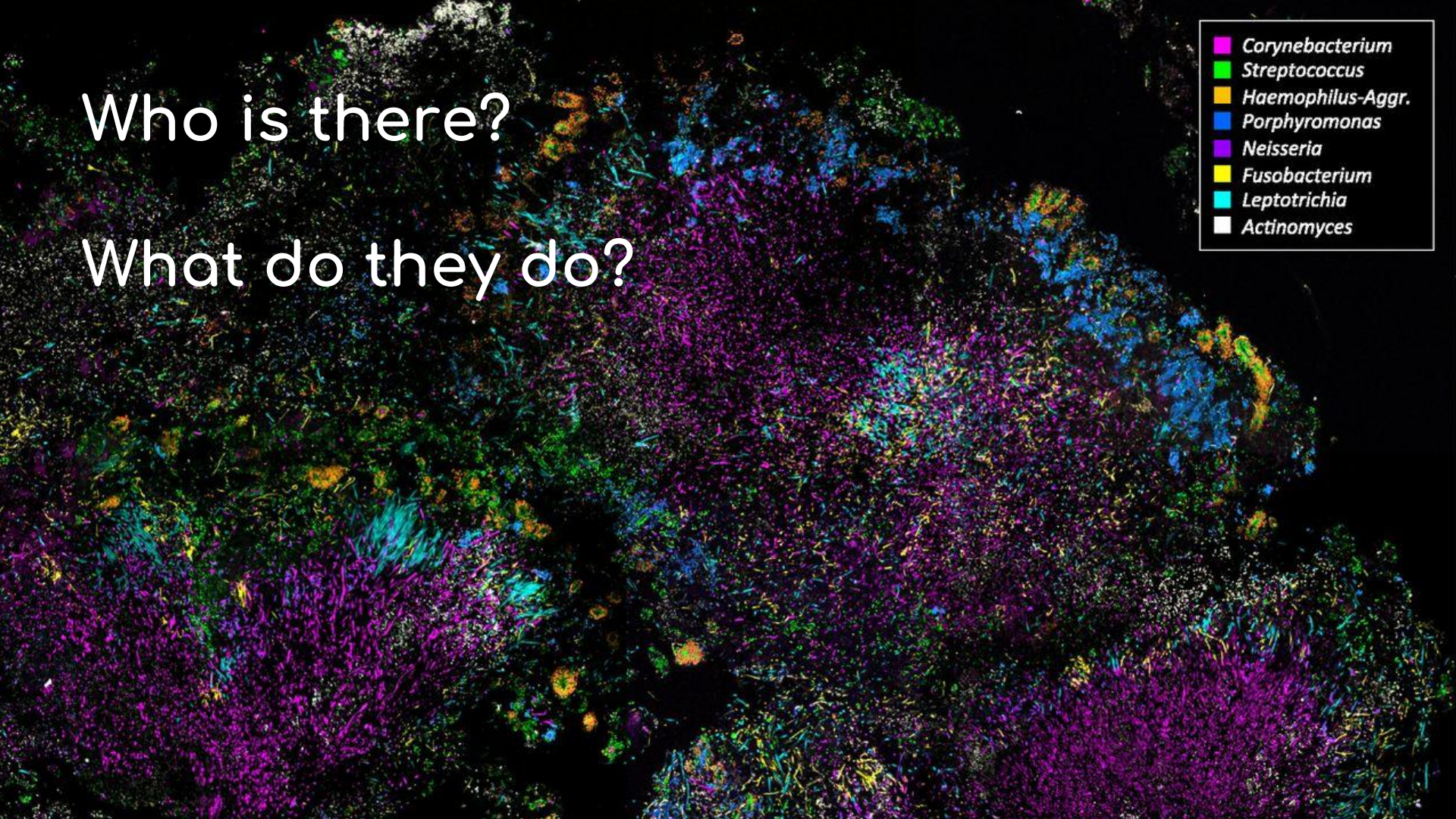
# Where are we?

| 3 weeks | 2 weeks | 2 weeks | 2 weeks |
| --- | --- | --- | --- |
| **From 16S sequencing to taxonomic profiles** | **From shotgun metagenomics to species- and strain-level taxonomic profiles** | **Comparing Taxonomic Profiles: Beta Diversity and Balance Trees** | **Inferring Species Interactions** |



# Outline

- General motivation
- 16S rRNA sequencing
- Full shotgun sequencing
- **MetaPhlAn**
- Results
- Conclusions & Discussion

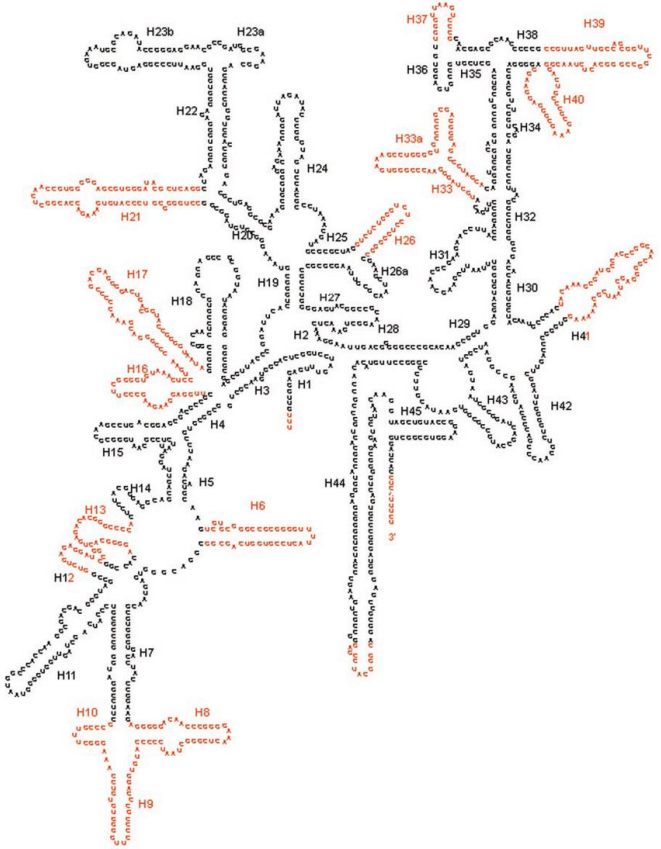



# Who is there?

# What do they do?

# 16S rRNA gene

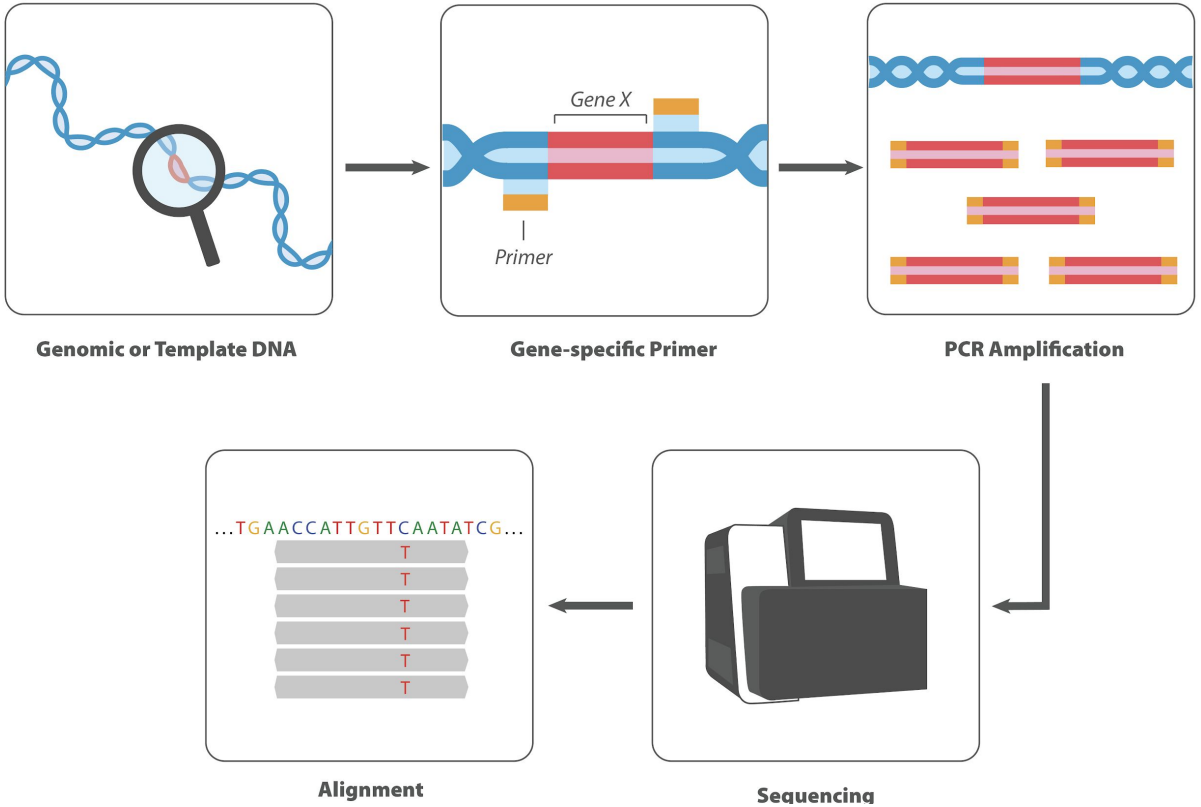
- Encodes part of the bacterial ribosome
- Present in all bacteria
- Very short usually (~1.5Kbp)



# 16S rRNA gene

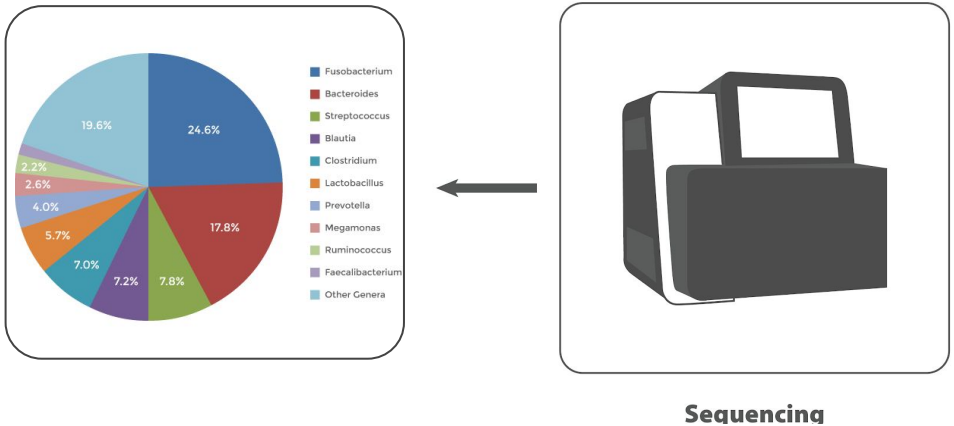
- Has conserved and **variable** regions
- Conserved areas → relationship among species
- Highly variable areas → differences between species

# 16S rRNA sequencing



# 16S rRNA analysis

- We saw different pipelines to determine:
  - Taxonomy
  - Relative abundances (RA)

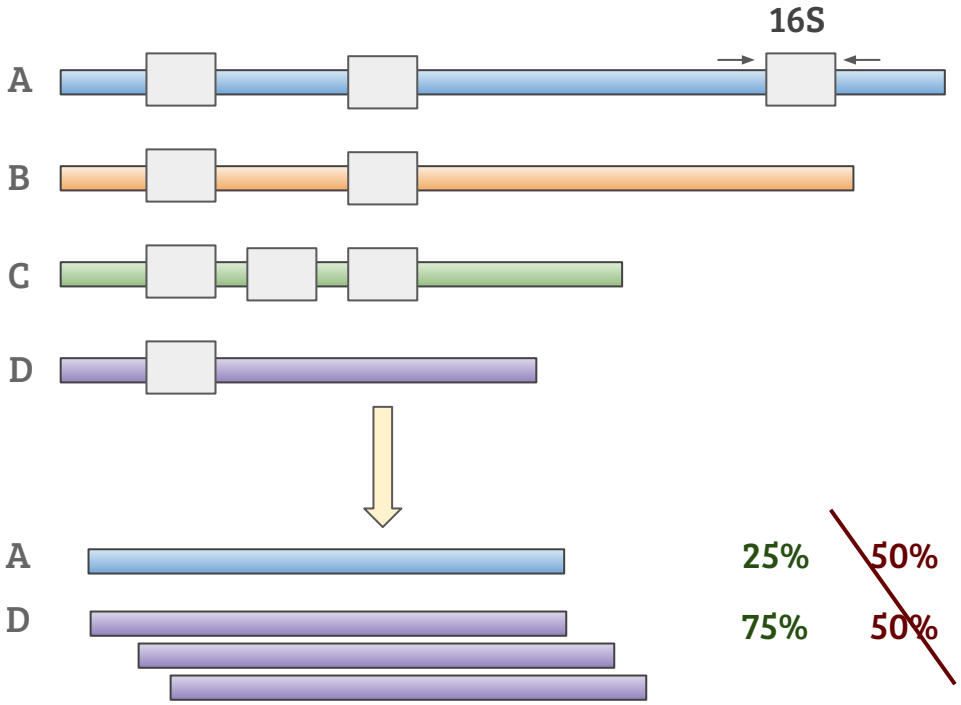


# Who is there? Solved!

- 16S technique seems to work great
- It’s solid, based on biological logic, and widely used

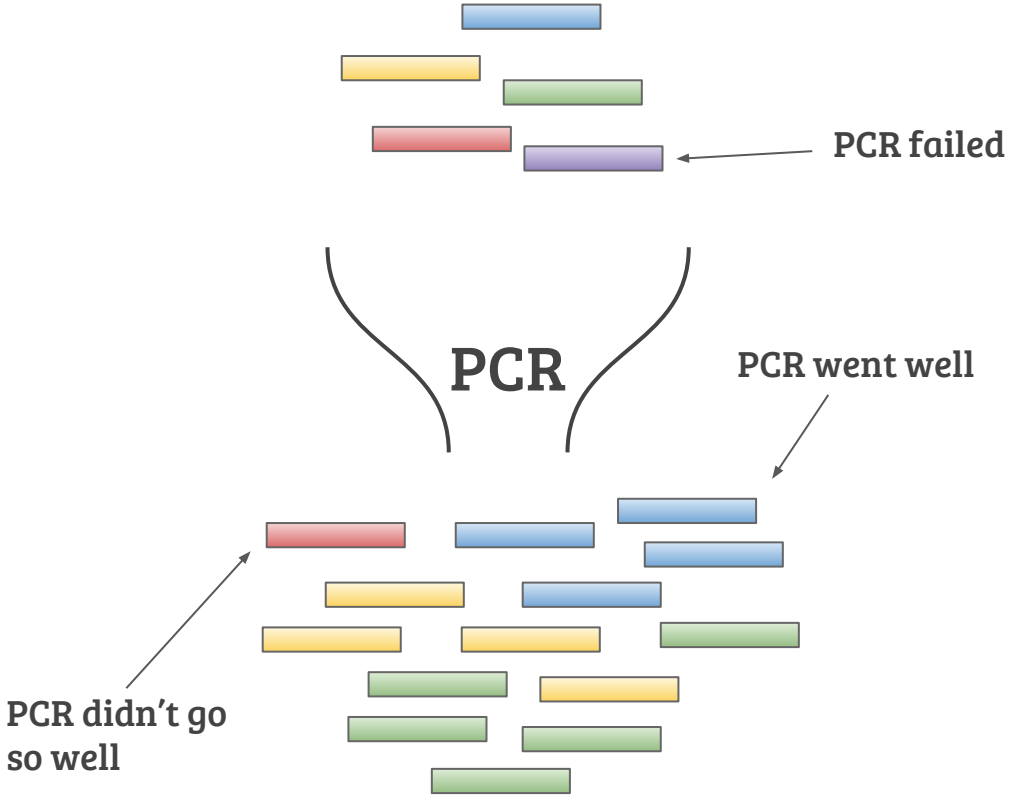
# Well not entirely...

- Copy number variation



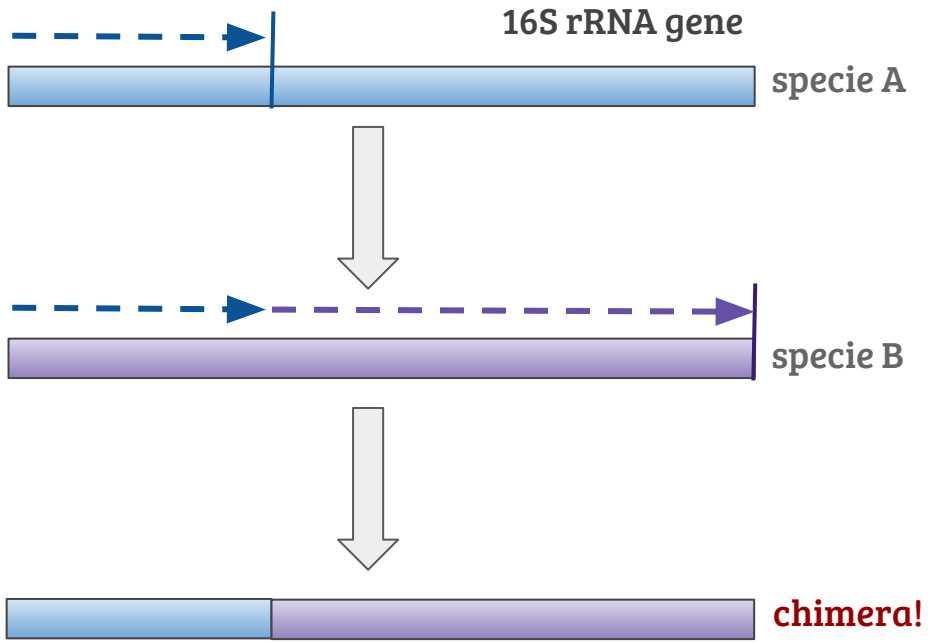
# Well not entirely...

- Copy number variation
- PCR amplification bias



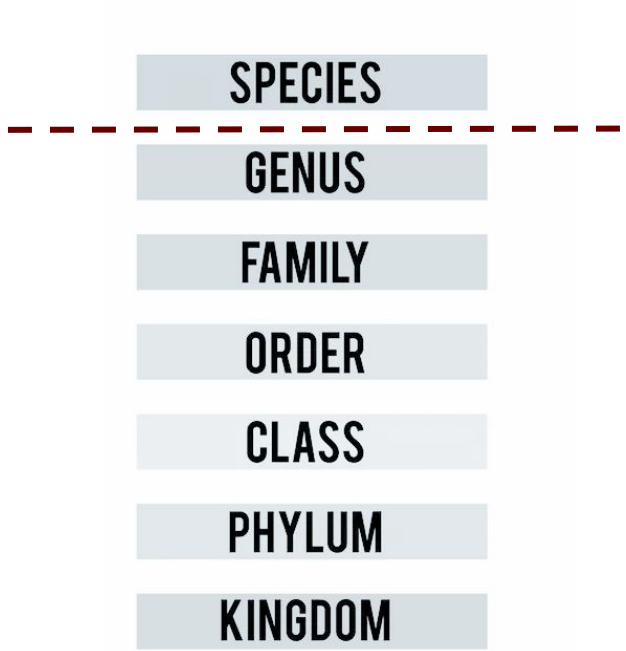
# Well not entirely...

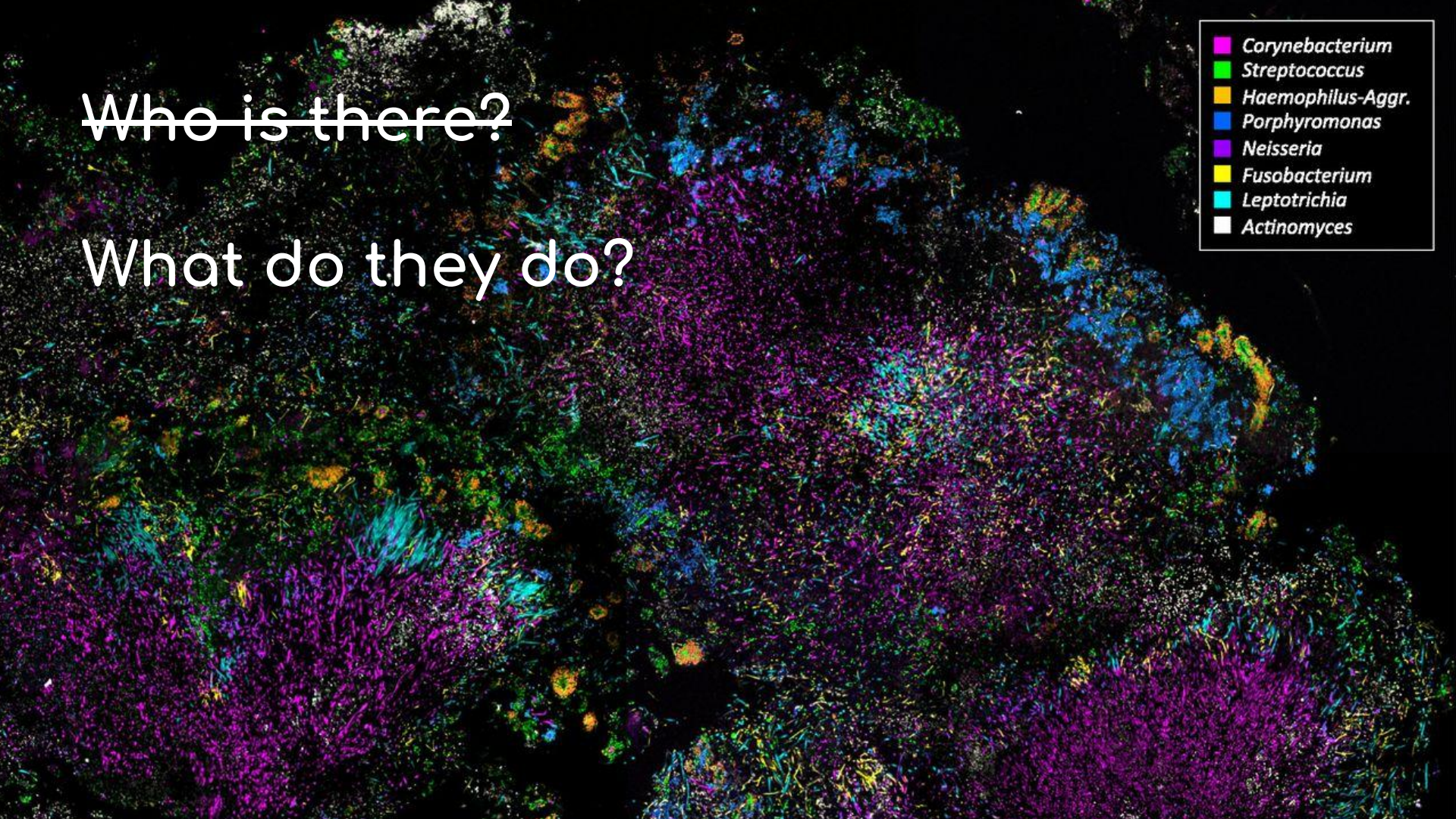
- Copy number variation
- PCR amplification bias
- PCR chimera formation



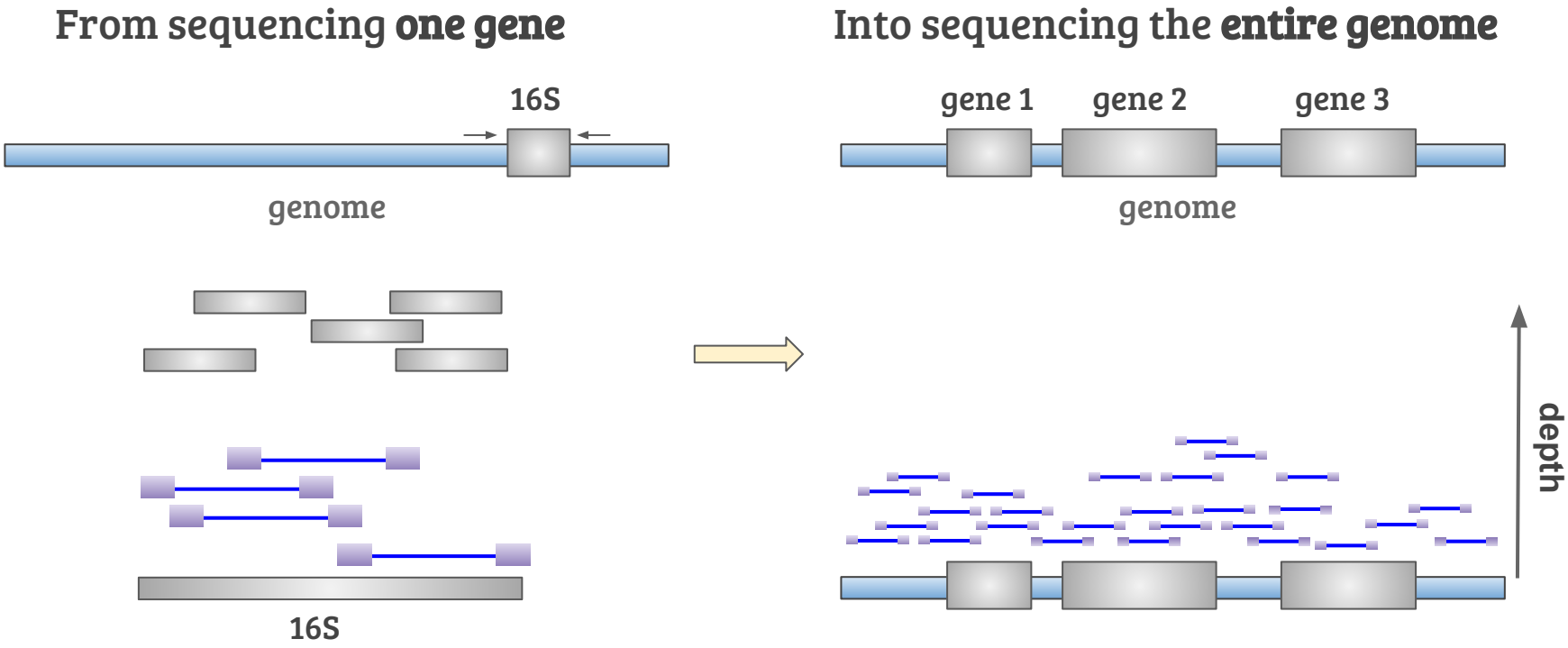
# Well not entirely...

- Copy number variation
- PCR amplification bias
- PCR chimera formation
- Limited resolution under genus




~~Who is there?~~
What do they do?
Corynebacterium
Streptococcus
Haemophilus-Aggr.
Porphyromonas
Neisseria
Fusobacterium
Leptotrichia
Actinomyces

# Full shotgun sequencing



# Full shotgun sequencing

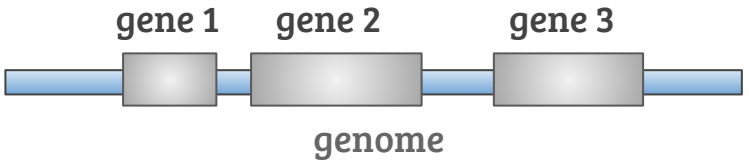
**Motivation**

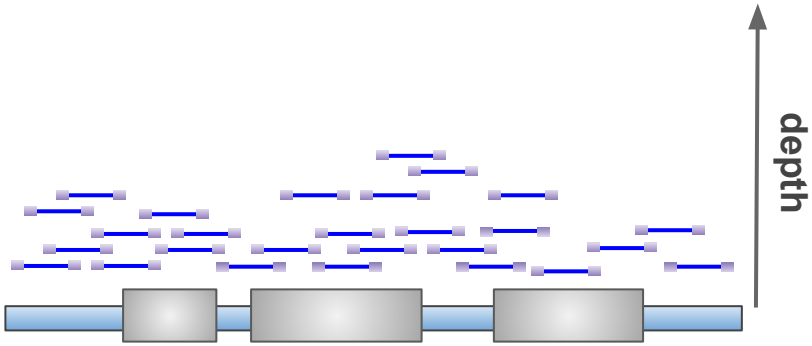
1. **Functional** information
   (e.g. *gene 1* is present)

2. Detecting **non-bacterial** species
   (can't use 16S rRNA gene)

3. Handling **"unseen"** genomes better

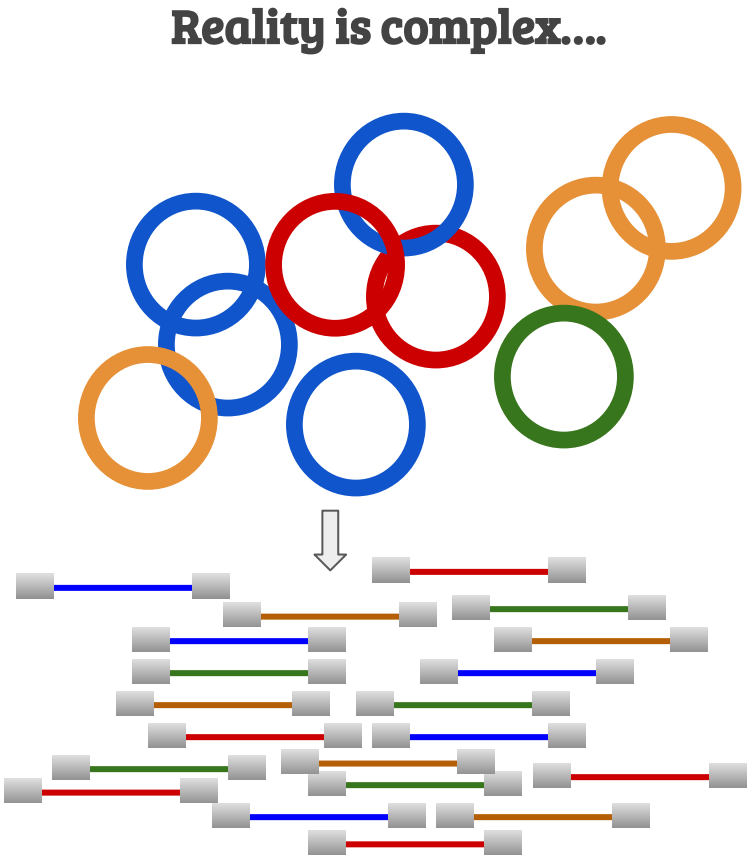
**Into sequencing the entire genome**



# Full shotgun sequencing

How can we tell “who is there”?



# MetaPhlAn (2012)

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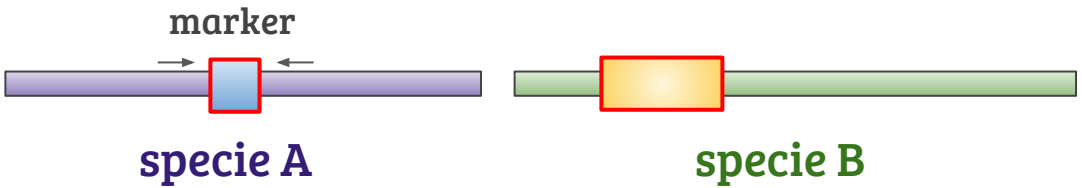
# MetaPhlAn - General intuition

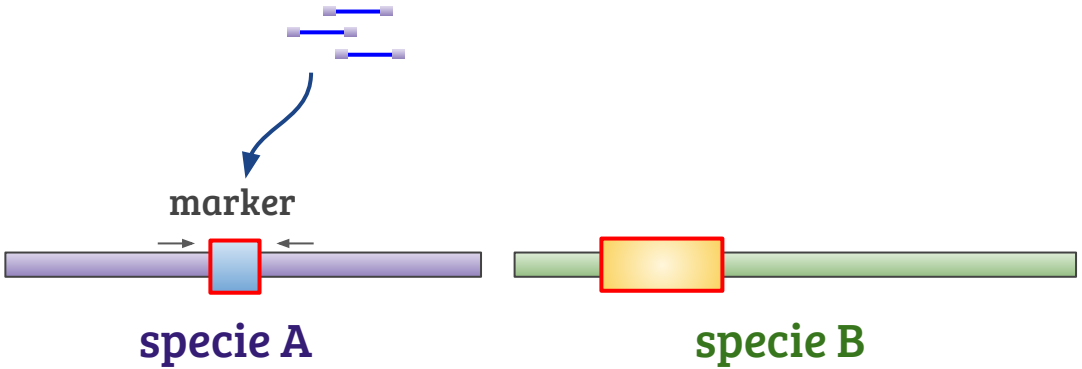
- We’re no longer limited to a single special gene

- Given the **full genomes** of our target species…
- We could look for “unique regions”



# MetaPhlAn - General intuition

- The region is “hit” by a read? → the specie is there!

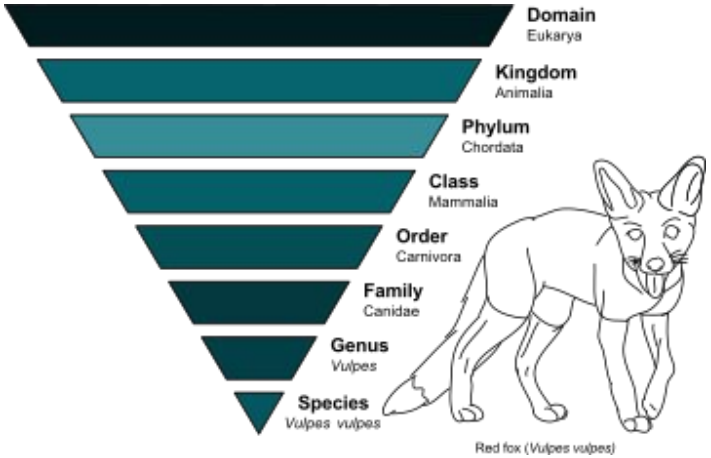



- We’ll call those regions *clade-specific marker-genes*

# Some definitions before we move on

- *Def:*
  **Clades** are groups of genomes (organisms) believed to have evolved from a common ancestor.

  It can be as specific as **species** or as broad as **phyla**.



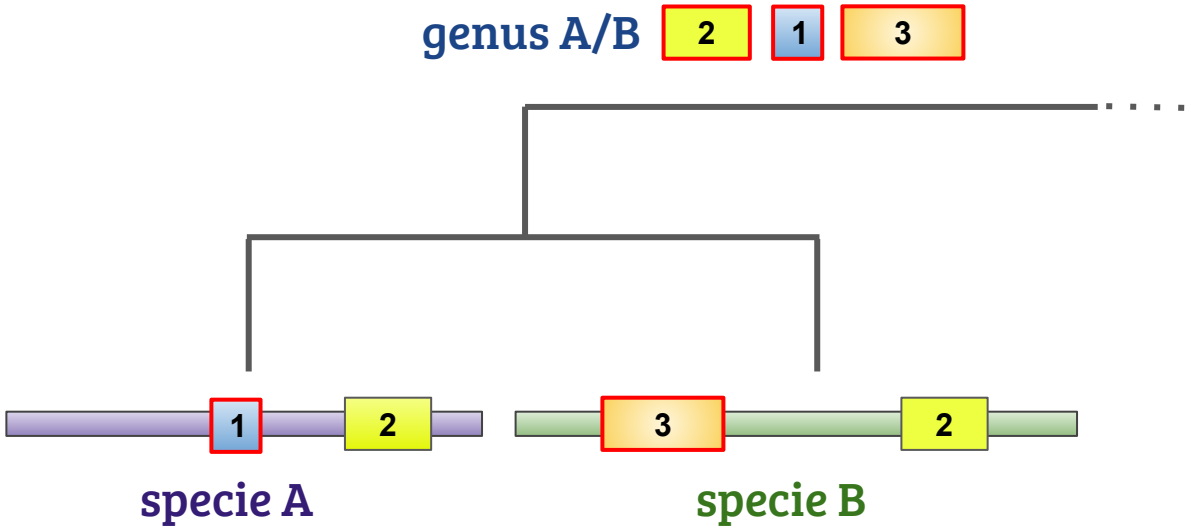
# Some definitions before we move on

- *Def:*
  **Clade-specific marker-genes** are sequences that satisfy:

  - Being **strongly conserved** within the clade's genomes.
  - **Not similar** to any sequence in other clades (of the same level)

# Clade-specific marker-genes

- Unique markers change as the clade level grows
- They also accumulate in a way (direct vs indirect)...



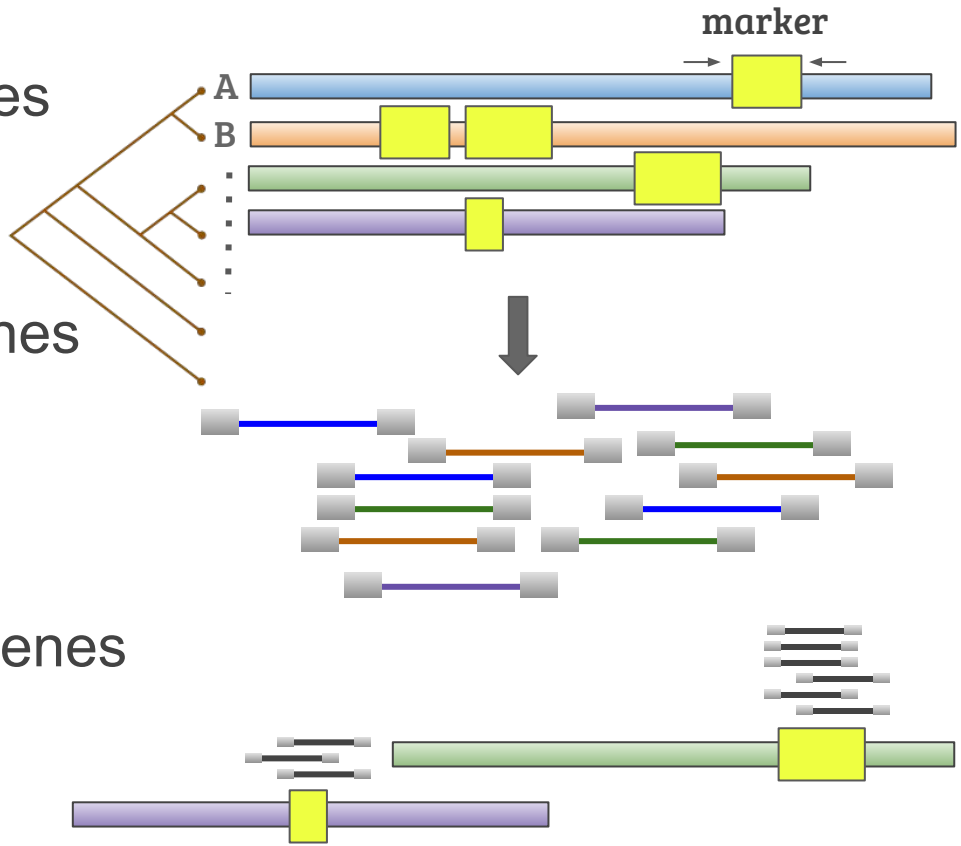
# MetaPhlAn - High level algorithm

- Use a set of reference genomes
- And a reference taxonomy

- Find clade-specific marker-genes

- Sequence sample

- Map reads to unique marker-genes
- Calculate “who is there”



# MetaPhlAn - High level algorithm

- Use a set of reference genomes
- And a reference taxonomy

Infrastructure

- Find clade-specific marker-genes

Offline

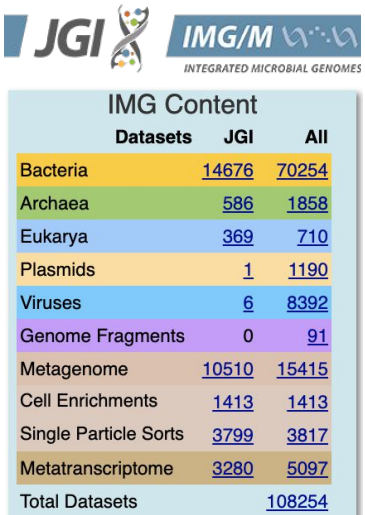
- Sequence sample

- Map reads to unique marker-genes
- Calculate “who is there”

Online

# Step 1: Acquire reference genomes

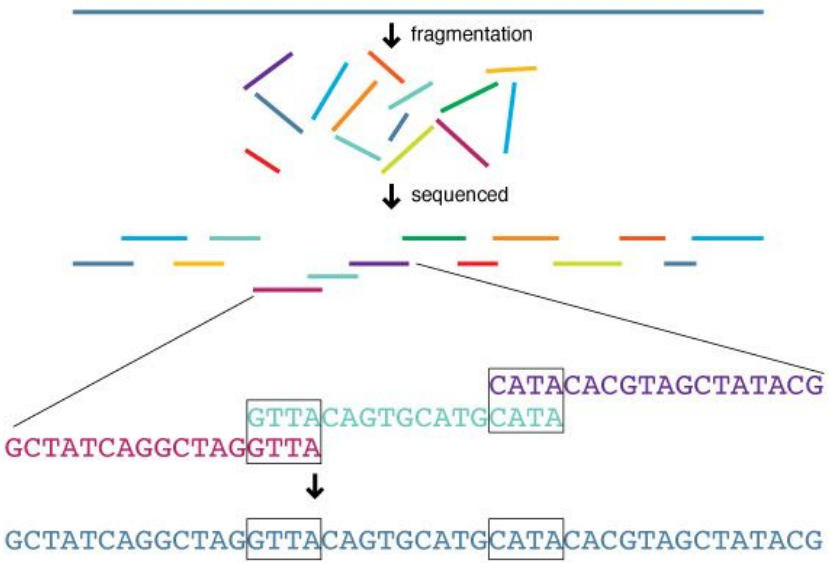
- **2,887 genomes** available from the Integrated Microbial Genomes (IMG) system
- The genomes are classified by quality measures
- They are based on many different public data sources (**non-homogenic!**)



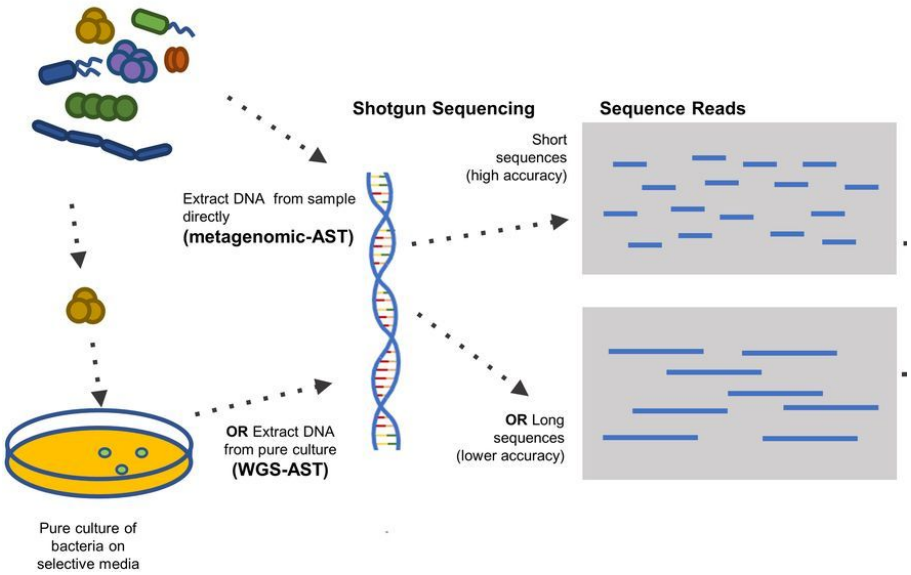
# Step 1: Acquire reference genomes

- The methods in which the genomes are acquired vary

***de-novo* assembly**

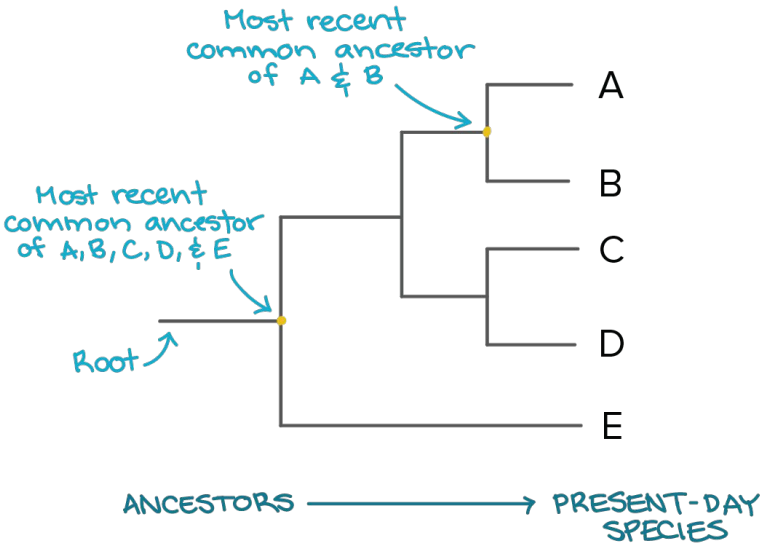



**cultured** species



# Step 2: Acquire reference taxonomy

- *The basic idea:*
  hierarchical clustering tree based on **genome similarity**



# Step 2: Acquire reference taxonomy

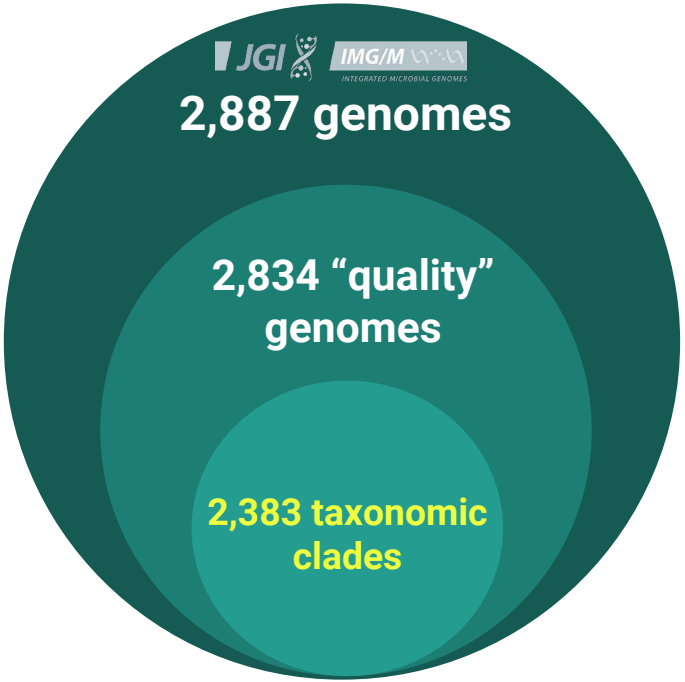
- *Wait...*
  Similarity of what sequences?

- Whole genomes are too long, too diverged

- Most databases still look for **evolutionary justifications** → distances are not based on the entire genome

- The 16S rRNA gene still plays a important part (!)

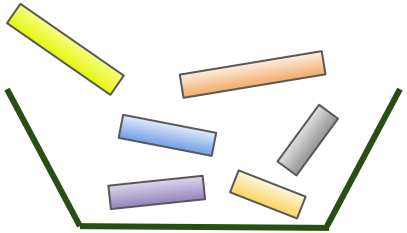
# Step 1 & 2: Acquire reference genomes & taxonomy

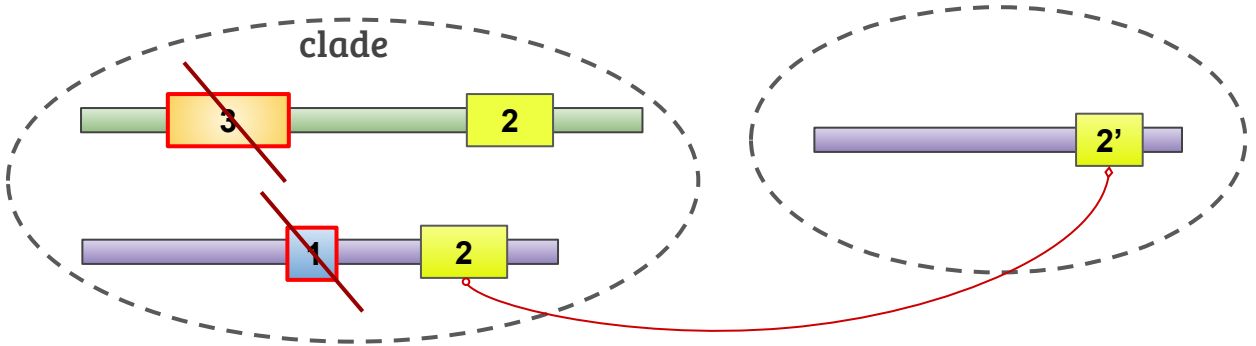



- ➔ 2 domains
- ➔ 33 phyla
- ➔ 66 classes
- ➔ 130 orders
- ➔ 278 families
- ➔ 652 genera
- ➔ **1,221 species**

# Step 3: Locate unique marker-genes

- The general process:
  - Each genome → bag-of-genes representation
  - Only conserved genes in the clade are saved
  - Inter-clade uniqueness index elimination
  - Single-copy genes were preferred of multi-copy genes





# Step 3: Locate unique marker-genes

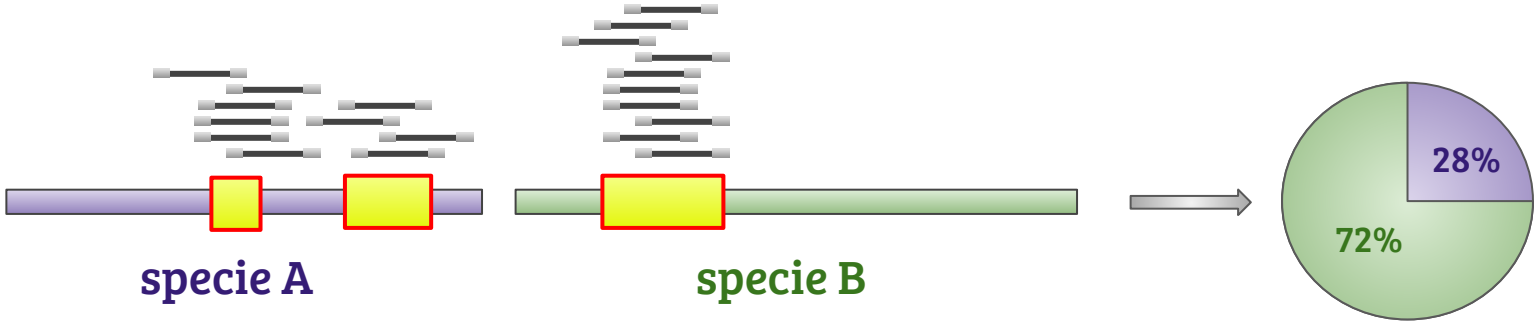
- Properties of the markers
  - **Gene** level
  - **400,141** filtered genes (out of original ~2M possible)
  - Not necessarily continuous (bag-of-genes)
  - **~4%** of the total genome length
  - **231** (± 107) markers per specie
  - Only 12 species with < 15 markers

# Step 3: Locate unique marker-genes

- Properties of the markers
  - As the clade grows (higher taxonomic levels)
    → it is usually **well covered**
  - Allowing MetaPhlAn to recover relative abundances within broader clades even in the **absence of sequenced genomes**

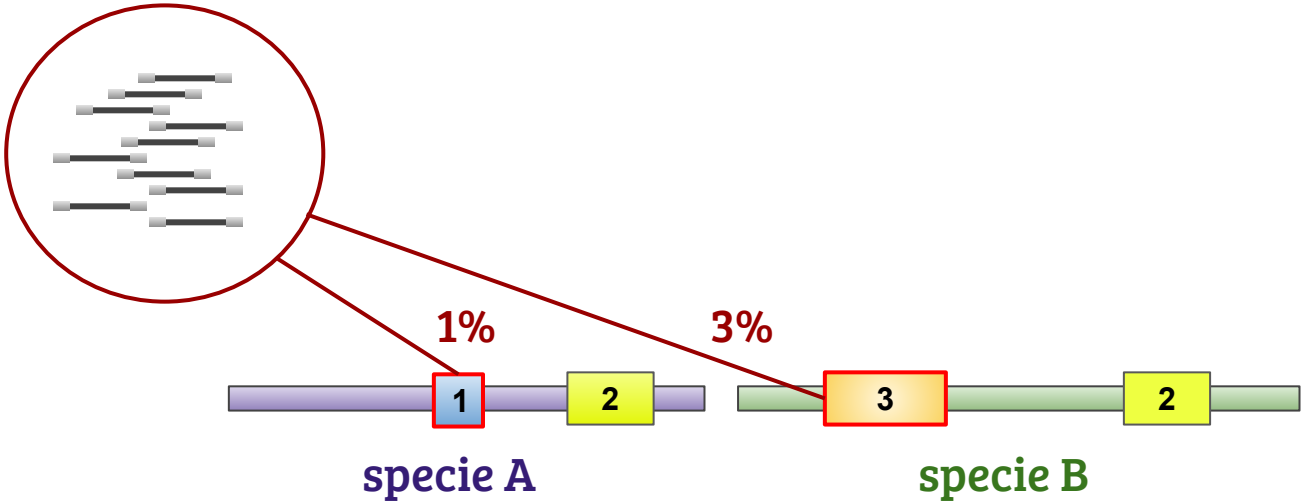
# Calculate relative abundances (who is there)

- Normalization
  - Sum the total reads mapped to clade markers
  - Divide by marker's total length
  - Abundances in every clade-level sum up to 100%



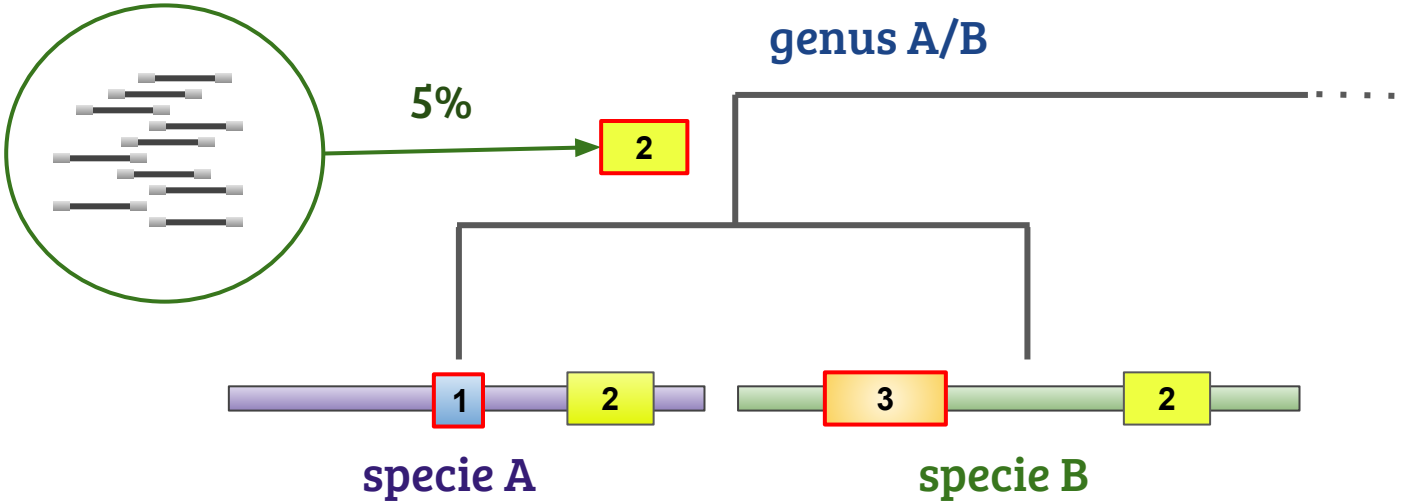
# Map reads to unique marker-genes

- The unclassified case:
  - Clade abundances obtained by direct read mapping



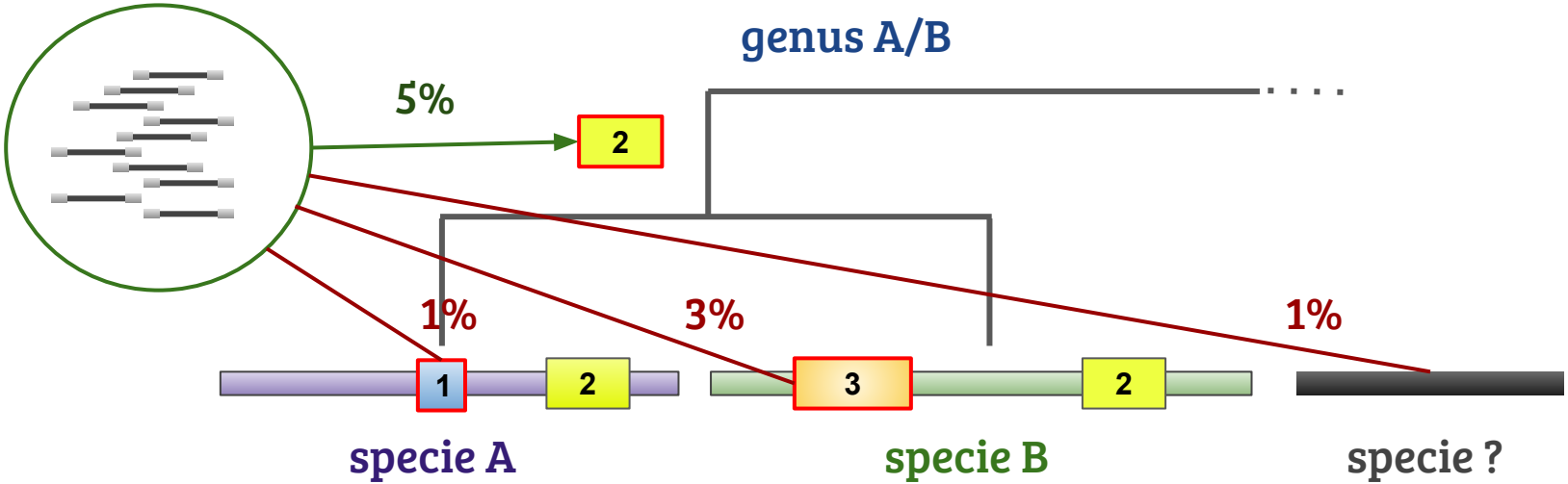
# Map reads to unique marker-genes

- The unclassified case:
  - The same is done to the clade at the next level



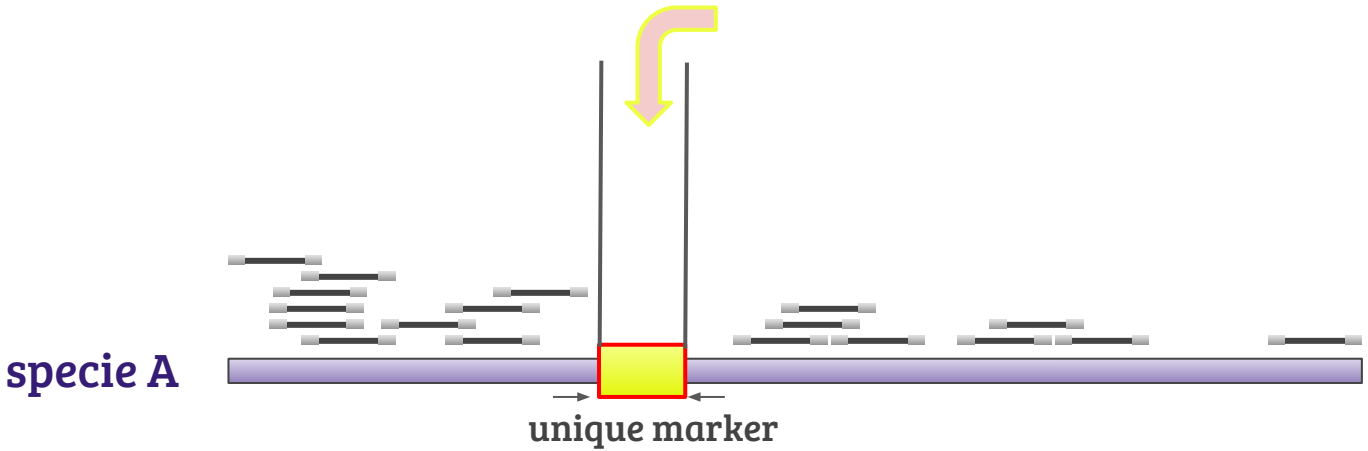
# Map reads to unique marker-genes

- The unclassified case:
  - What if there is a contradiction?



# Limitations

- Balls into bins problem

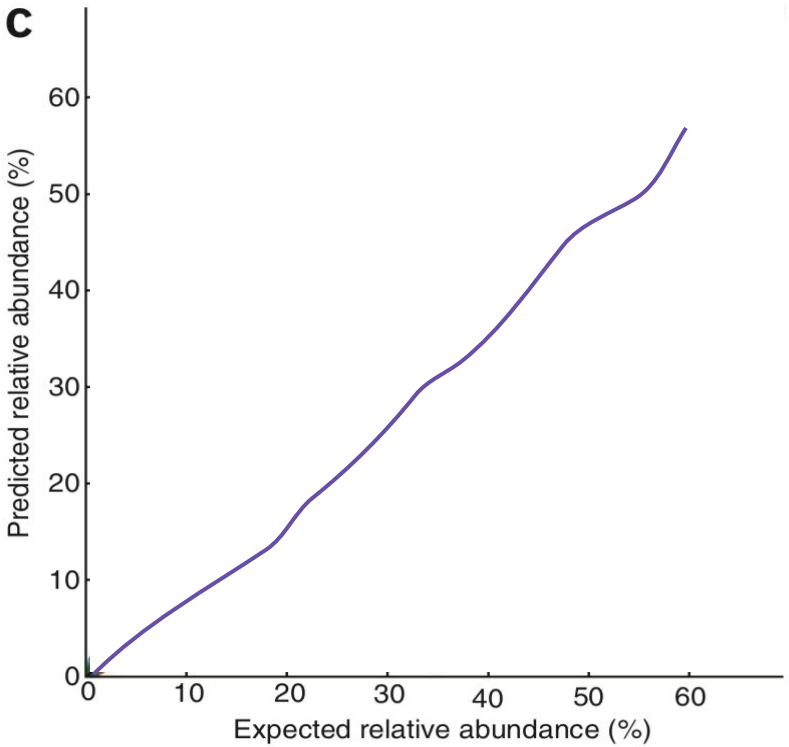



- Happens in low abund. (theoretical prob. can be calculated)
- MetaPhlAn is known to operate poorly at low abundances

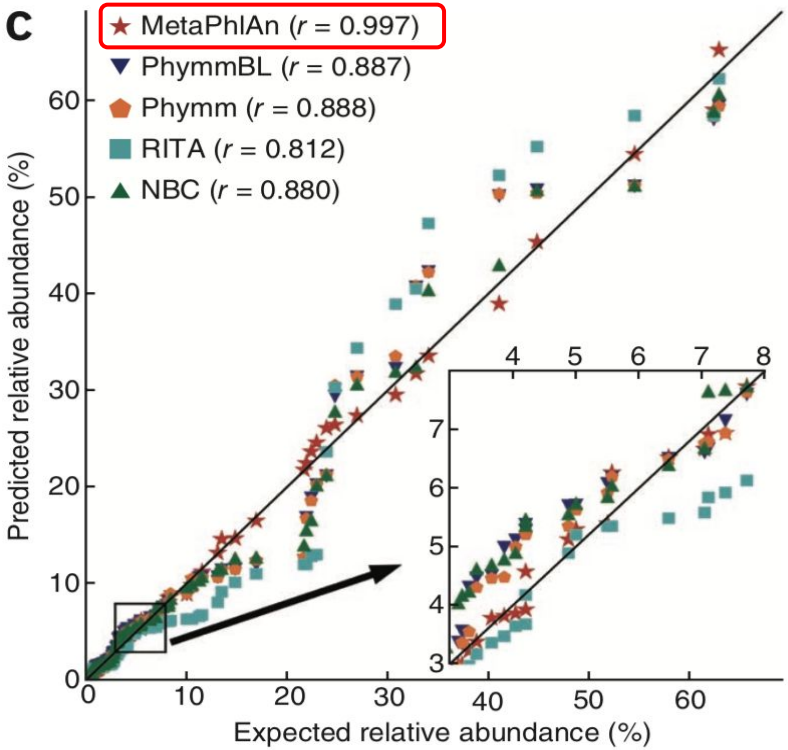
# Estimating performance

- Synthetic communities
  - 2 samples with 100 genomes each (**high-complexity**)
  - 8 samples with 25 genomes each (**low-complexity**)
  - Total of 4M synthetic noisy reads

# Estimating performance

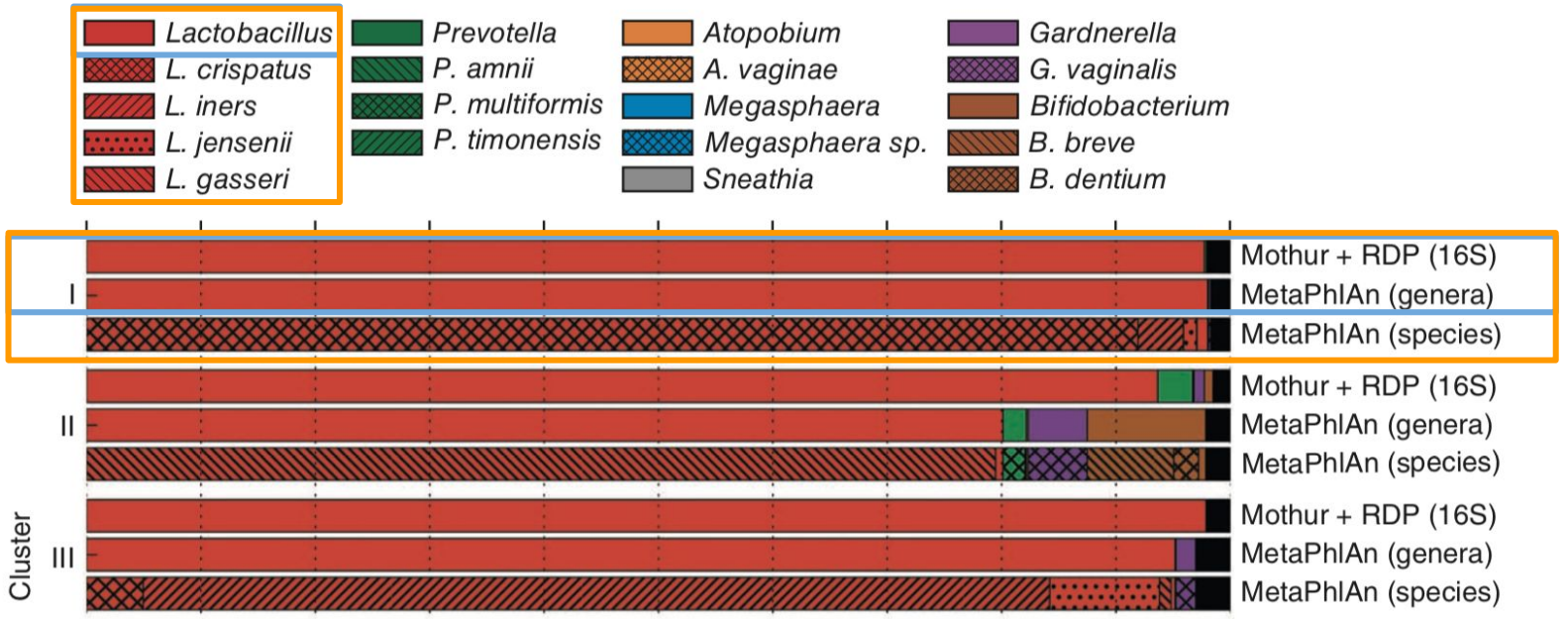


# Estimating performance



# Estimating performance

## higher resolution compared to 16S



# Conclusion

- Reality is complex. There is no one solution.
- All methods have internal biases and limitations

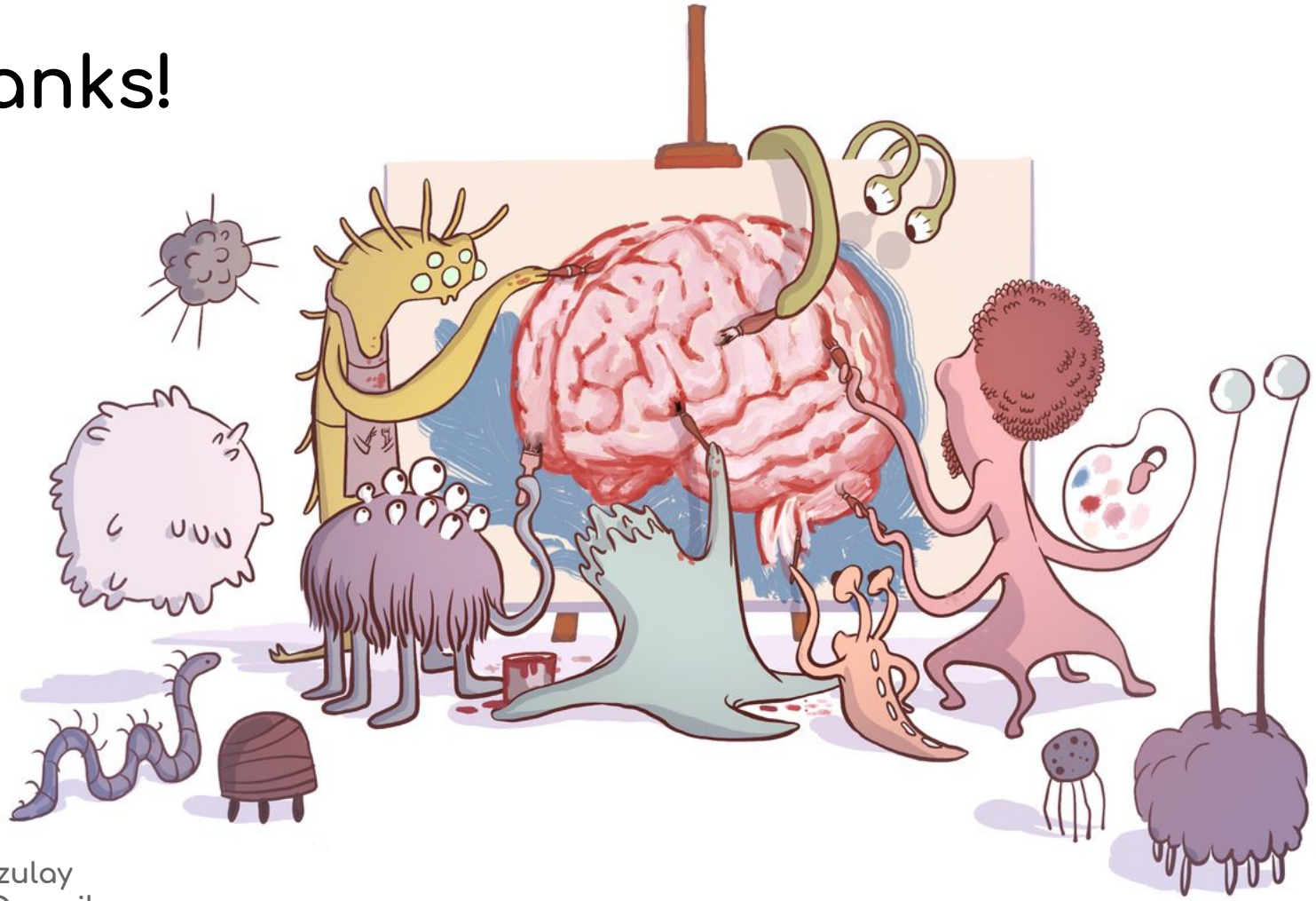
- MetaPhlAn sets a good standard in many settings.
- It is commonly used, and very well known.

- These are still areas of active research, and progress is still at a steep curve.

# Points for discussion

- What are the biggest generators of noise in the process?
- What happens when the true species skewes from the ref. genome?
- How can I know what sequencing depth I should use?...

# Thanks!



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