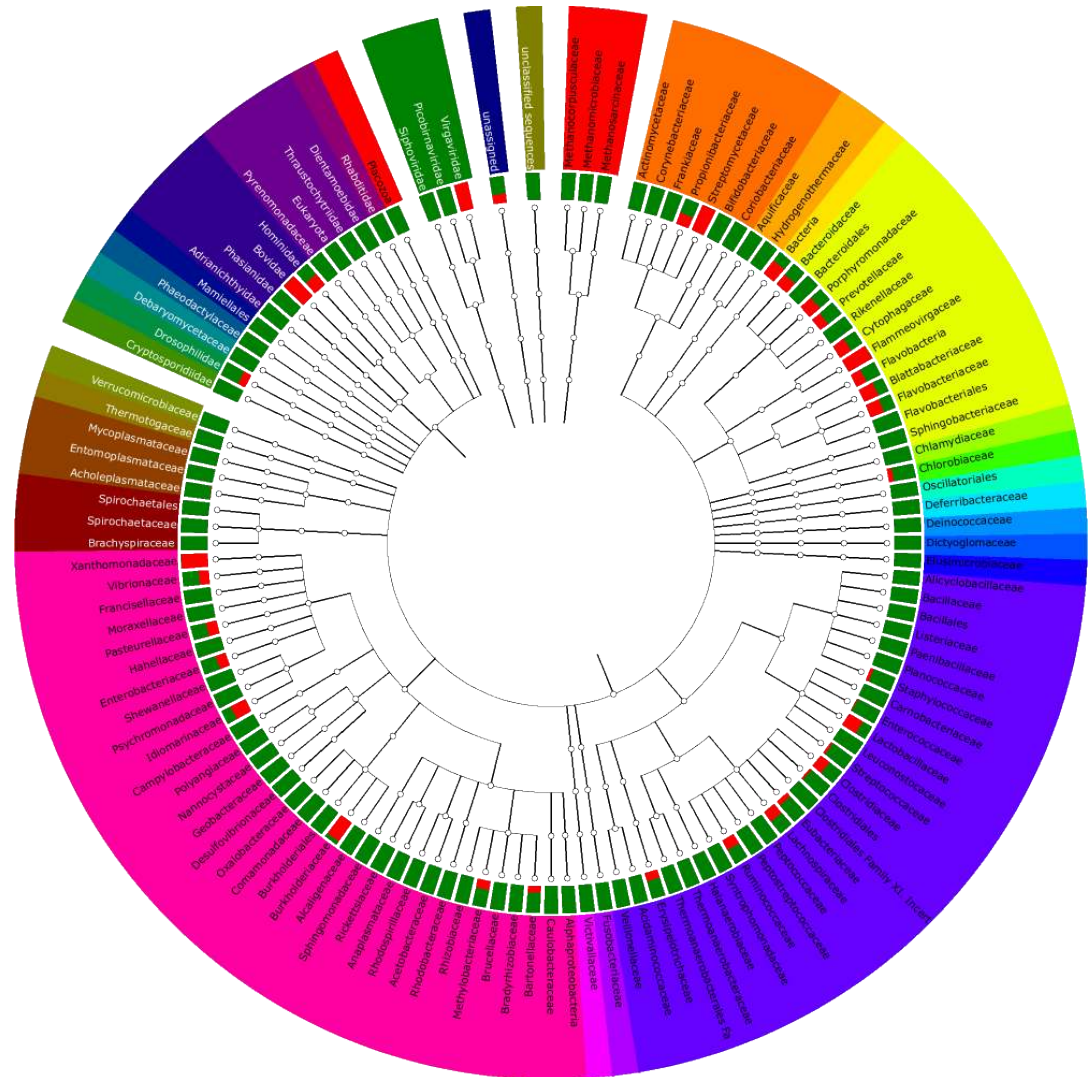


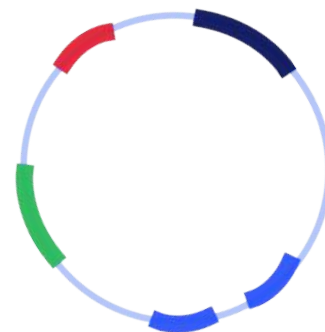
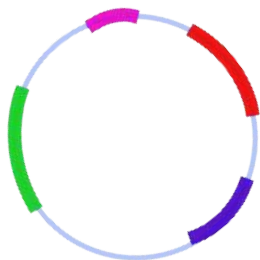
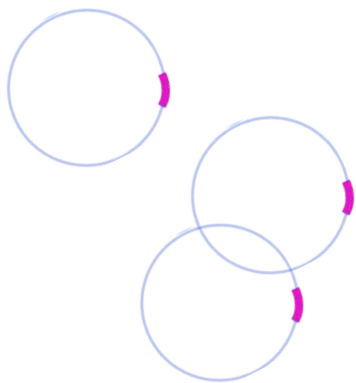
Metagenomic Analysis

Outline

- Gene targeted vs. random approaches
- Diagnostic versus ecological uses
- Case study
- mg-RAST
- MEGAN demonstration

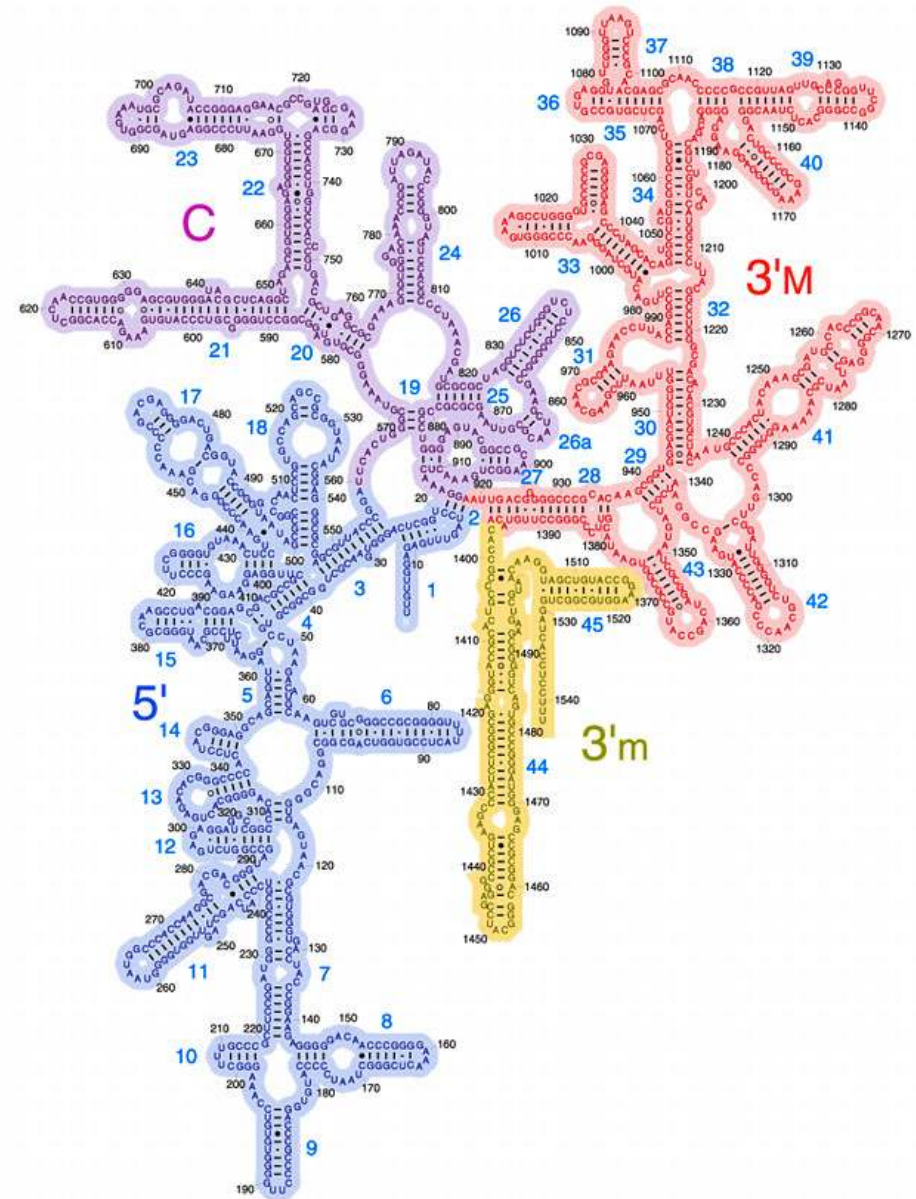


Gene Targeted and Random Shotgun Approaches

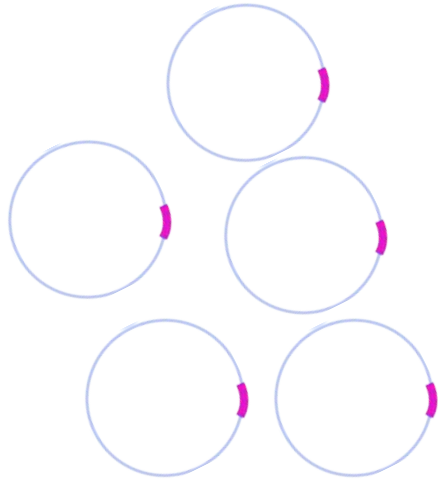


16s rRNA

- a section of prokaryotic DNA found in all bacteria and archaea
- encodes the small subunit of the ribosome
- 1.5 kb
- conserved and hypervariable regions
- used for decades in clinical microbiology and studies on bacterial evolution
- used extensively in metagenomic studies



targeted - 16s rRNA

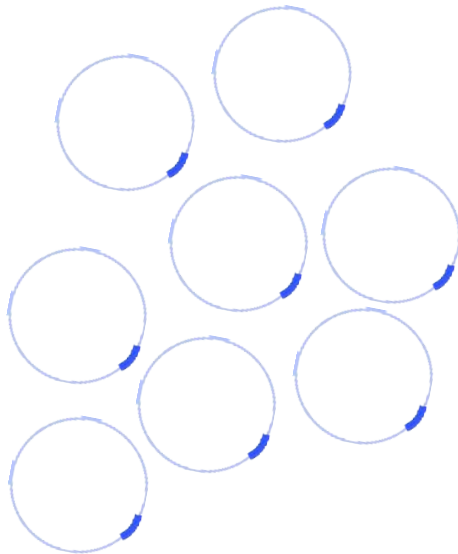


CTACAATGTGTTCTGTACGTACGGCACTTTTCNGCATTCTGACAGTACG
CTACAATCTGTACGTACGTTCGGCACTATCNGCATACTGACAGTACG
GTACAATGTGTACGTACCTACGGCACTATCNGTATACTTCCAGCACG
GTACAATGTGTACGTACGTACGGCACTTTTCNCCATACTGACAGTACC
CTACATTGTGTACGTACGTACGGCACTATCNGCATACTGACACTACG

Operational Taxonomic Unit

Database search

Escherichia coli



AACTGTCCTCTGGTATGCACGGCACTTTTTGCATACTGACAGTACGA
ATCTCTCCTCTGGTATGCACGGCACTTTTTGCATACTGACAGTACGA
AACTGTCCTCTGGTATGCACGGCACTTTAAGCATACTGACAGTACGA
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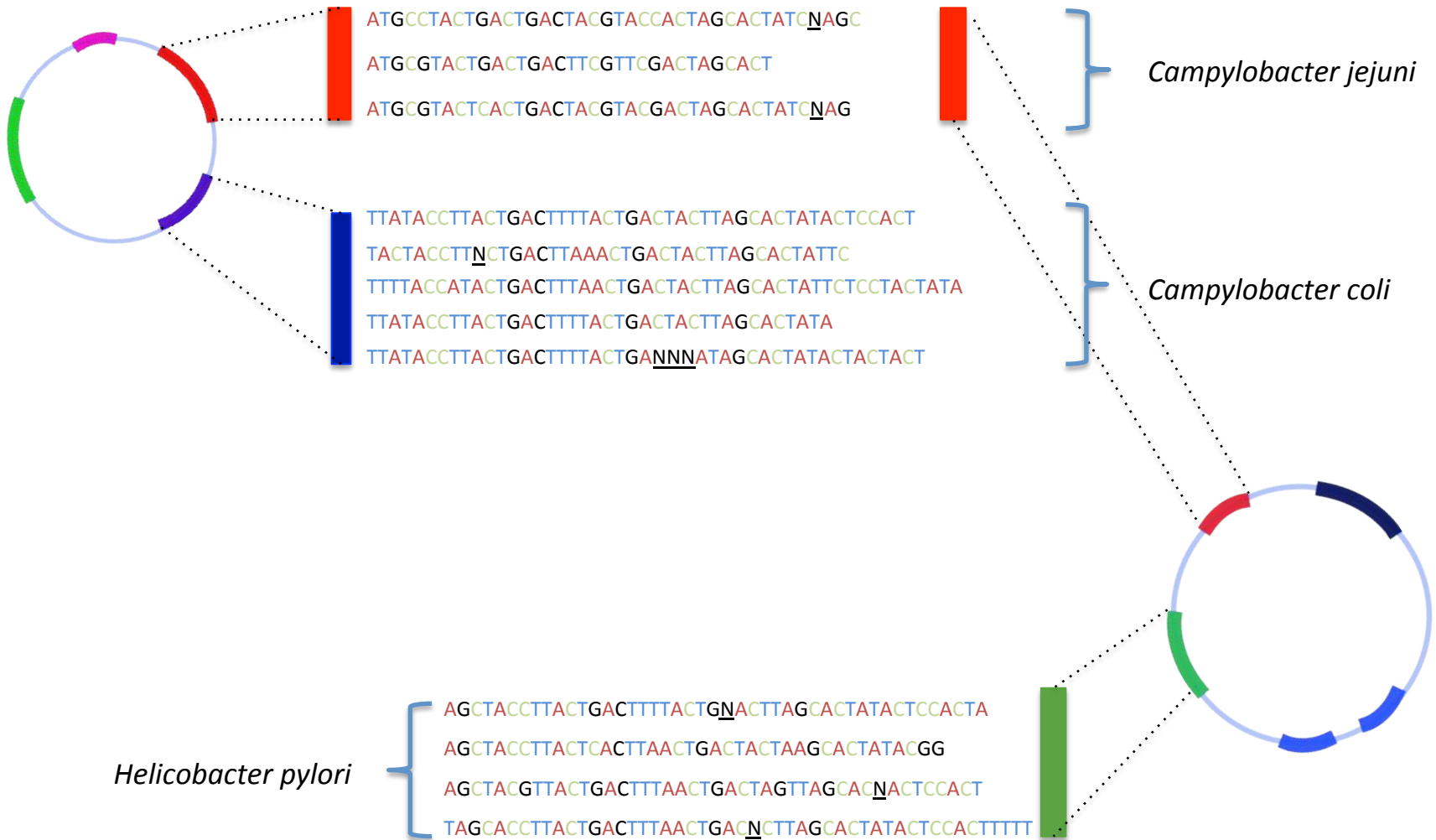
Operational Taxonomic Unit

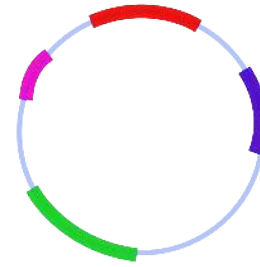
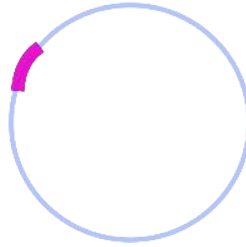
Database search

No hits

Not *Escherichia coli*

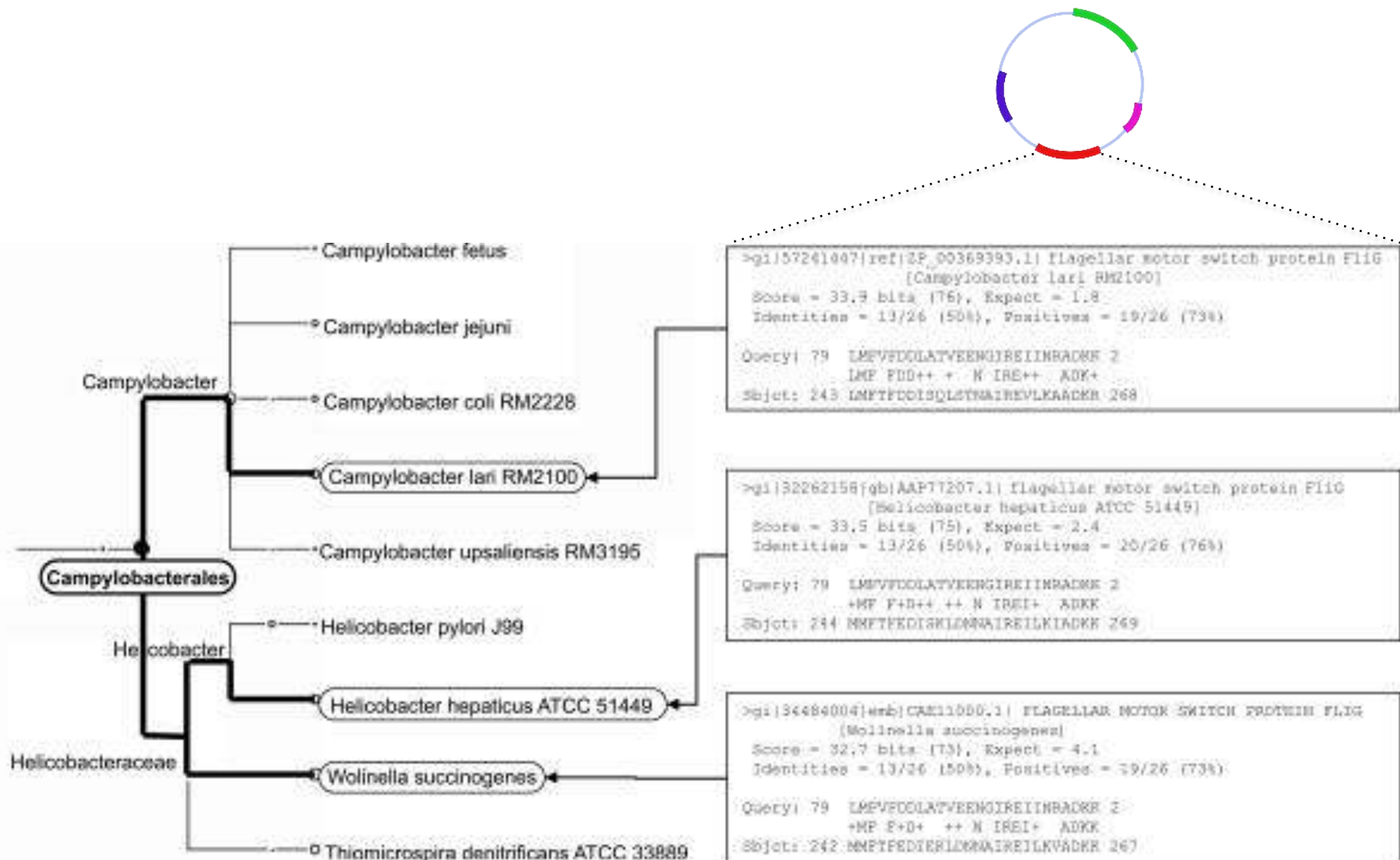
Random sequencing



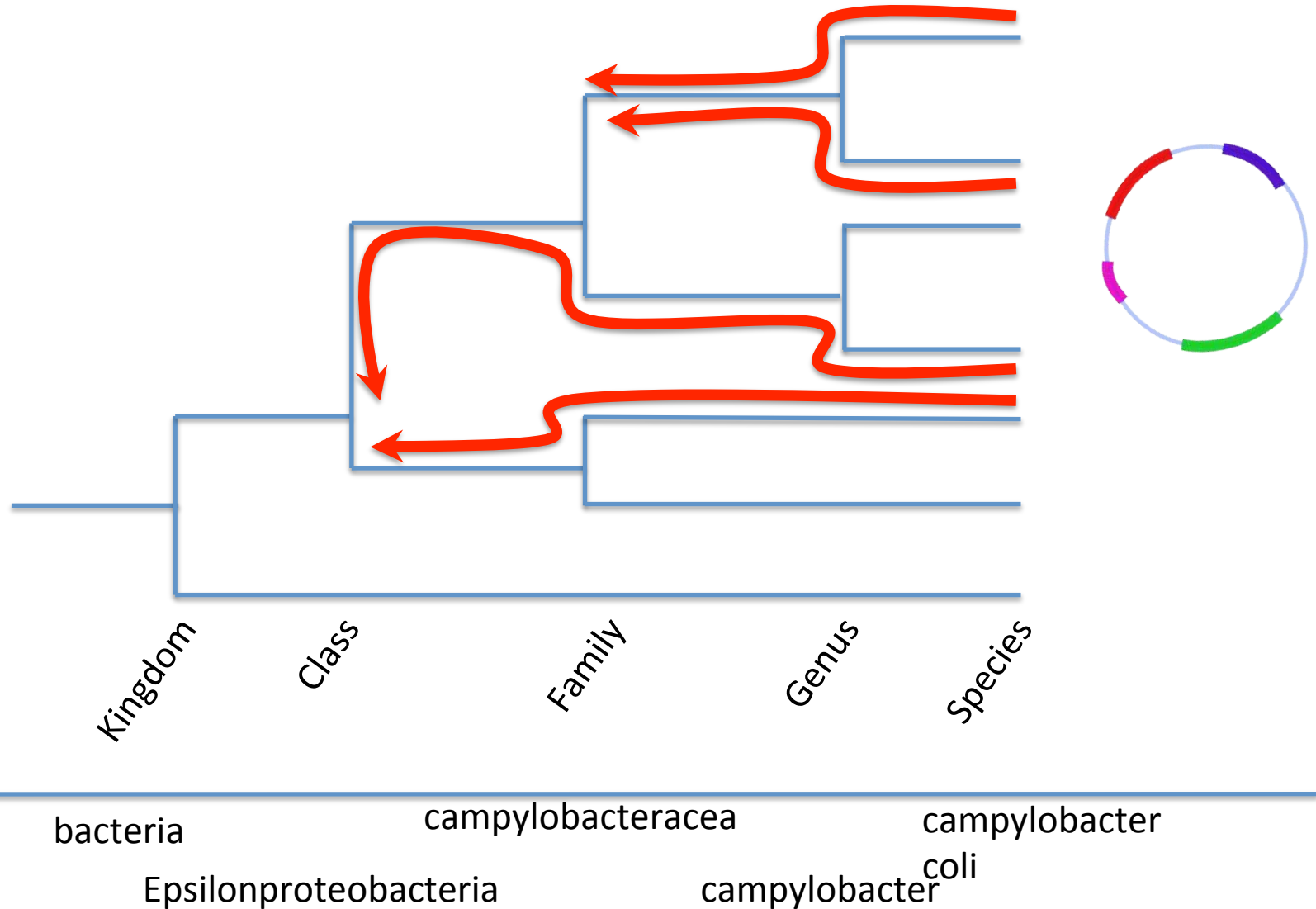


	16s rRNA	Random
Read quality	High	Variable
Reference Database	Good	Variable / Potentially more biased
Taxonomic Assignment	Partially database independent	Database dependent
Non-bacterial assignment	Impossible	Database dependent
Phenotype assessment	Assumed	Unknown

Lowest-common ancestor (MEGAN)

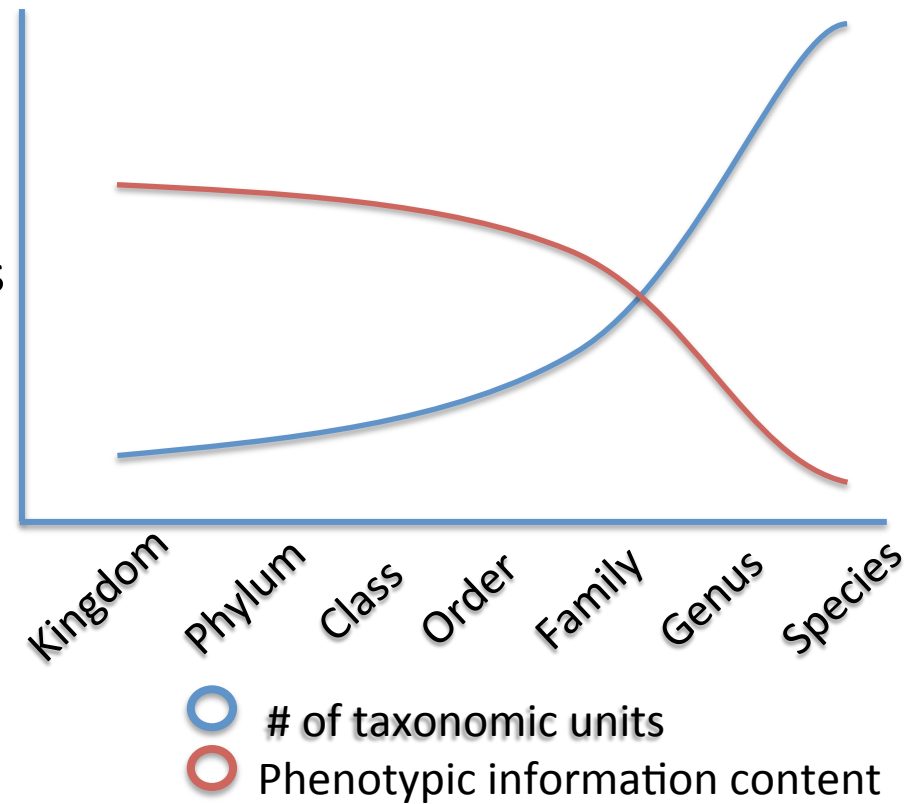
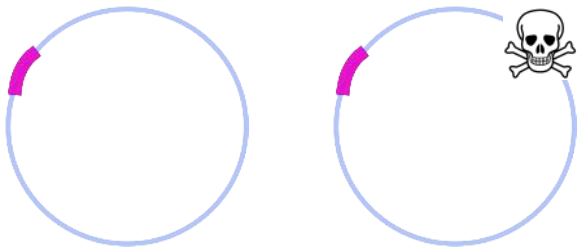


Assignment tends to move up the tree

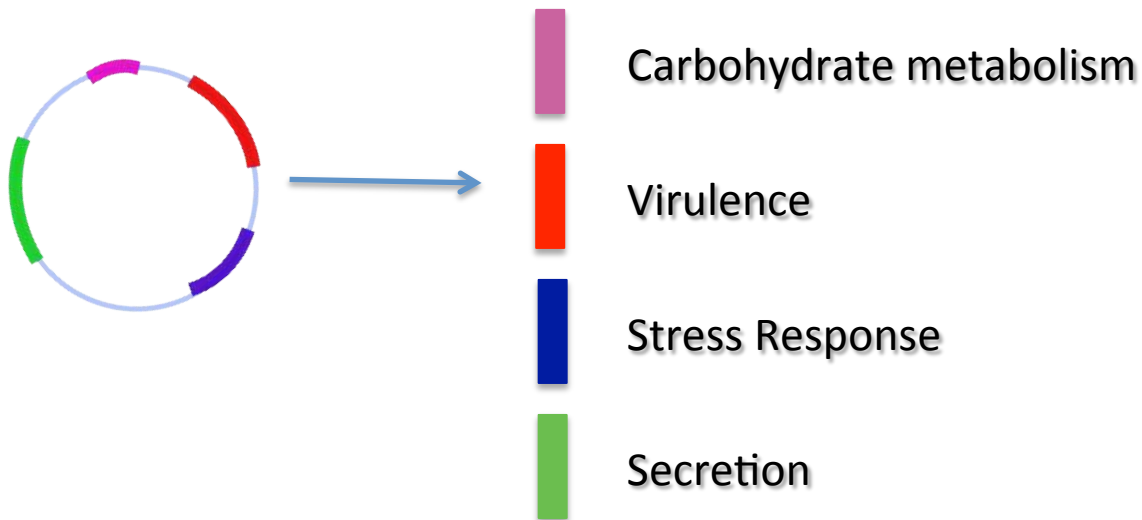
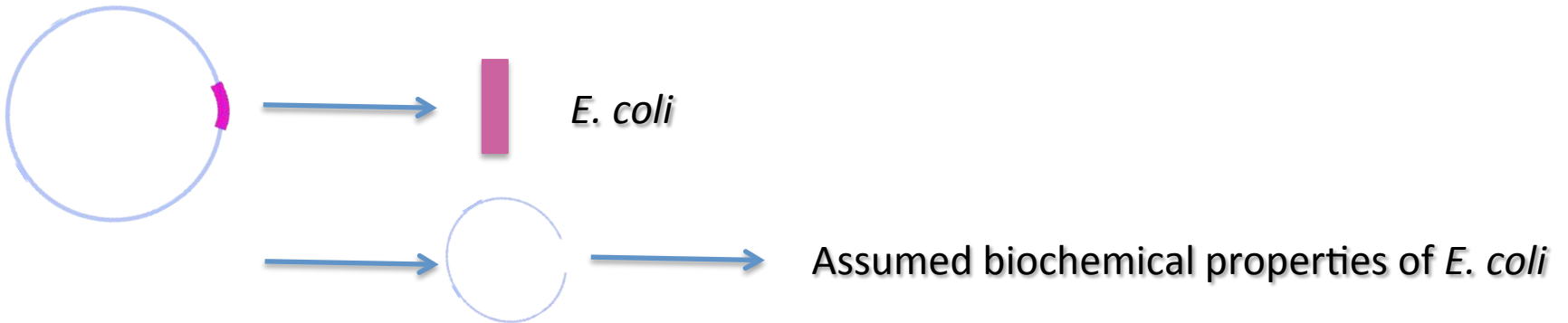


What level of taxonomic resolution do we need?

- Depends on what you want
- Diagnostics vs. ecology
- The functional ecology promise of metagenomics might not benefit from species level analysis

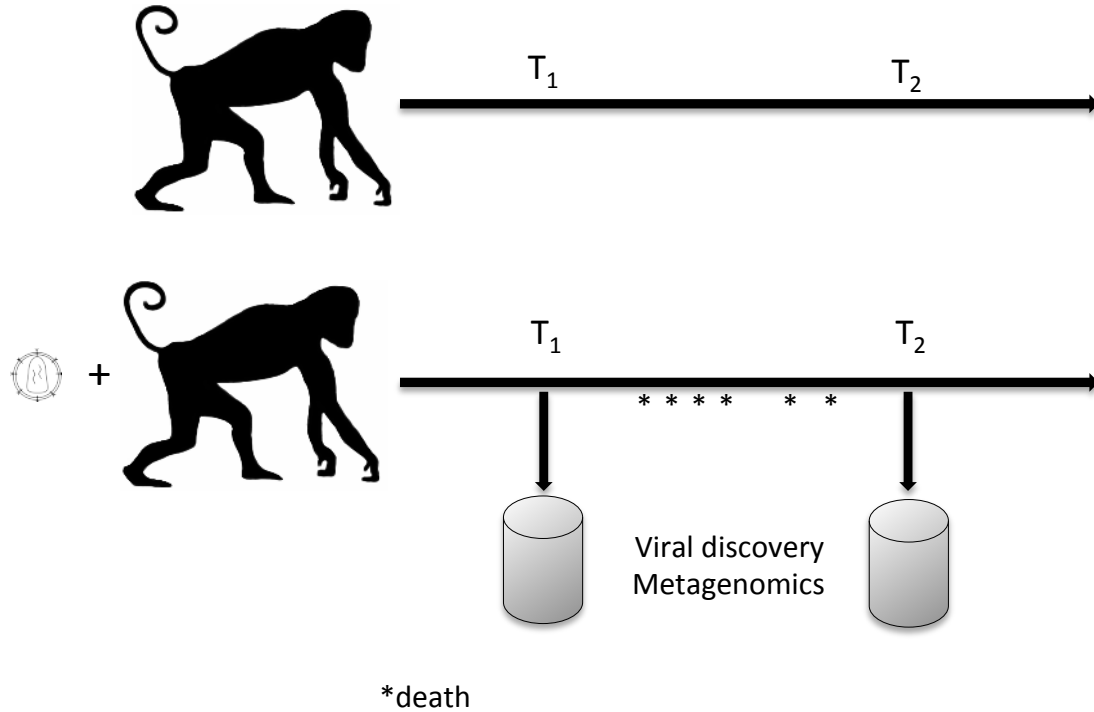


16s vs. random

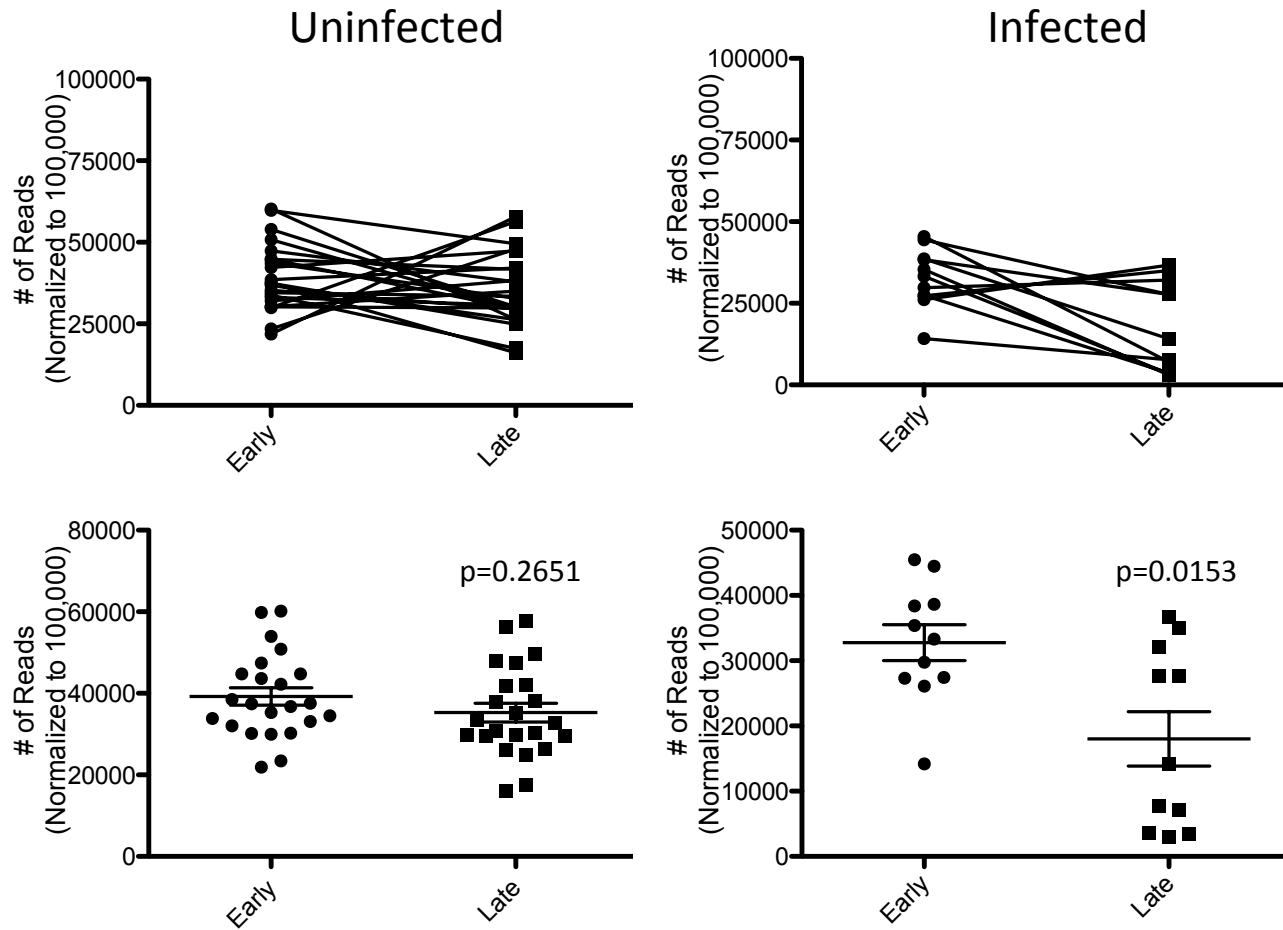


SIV Macaque Gut Metagenomics

SIV Study Cohort

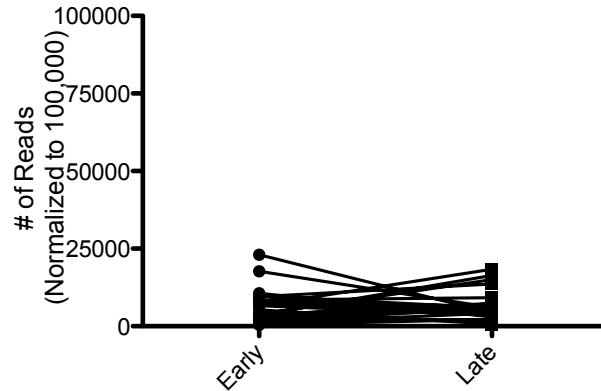


Bacteria

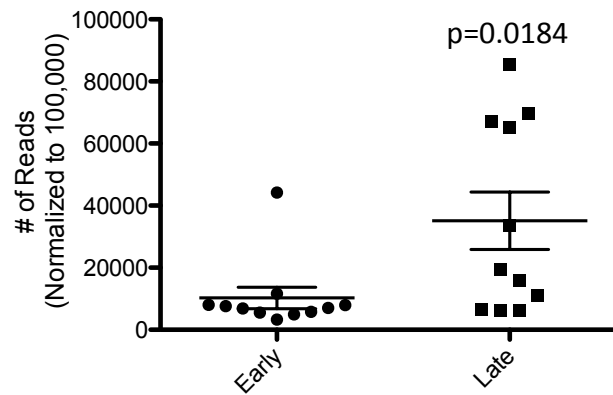
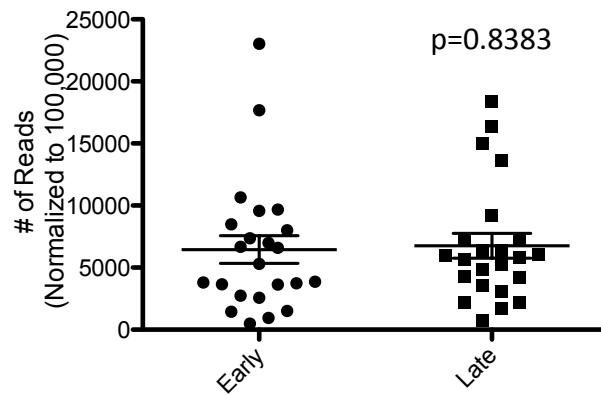
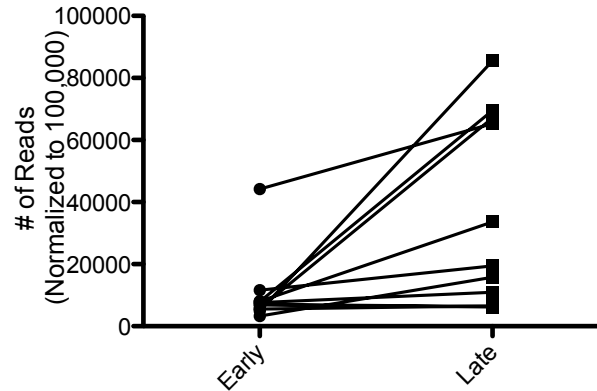


Virus

Uninfected

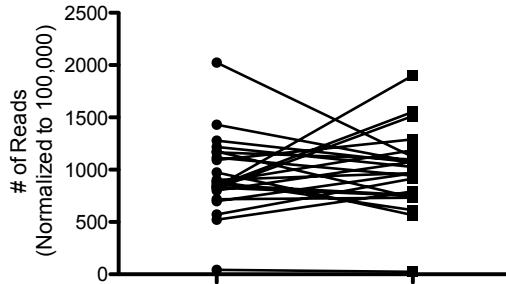


Infected

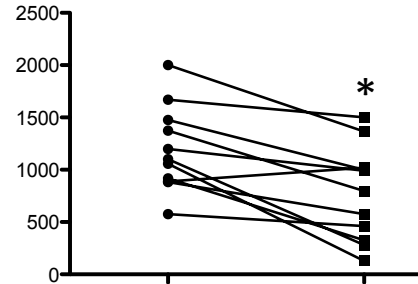


Other Kingdoms

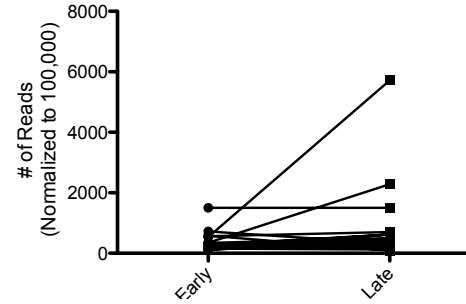
SIV (-) Fungi



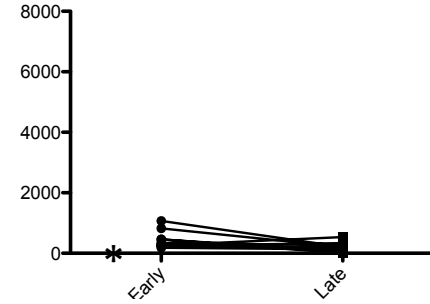
SIV (+) Fungi



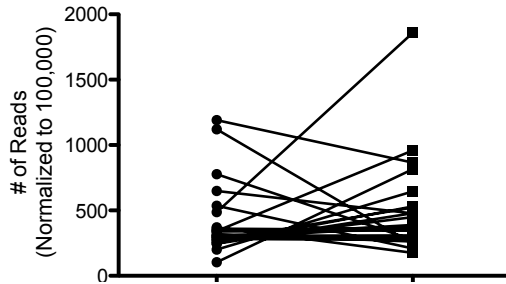
SIV (-) Amoebozoa



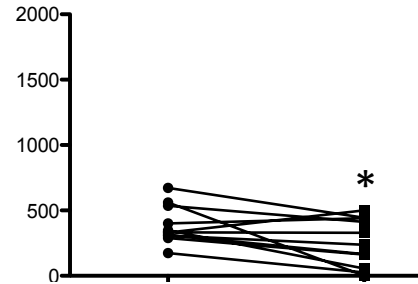
SIV (+) Amoebozoa



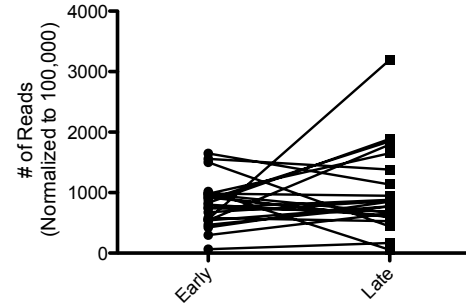
SIV (-) Archaea



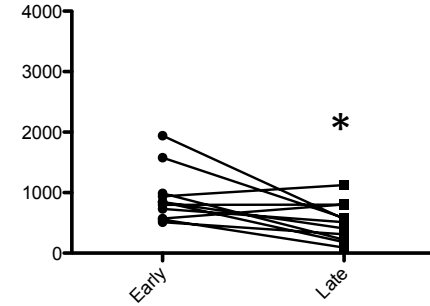
SIV (+) Archaea



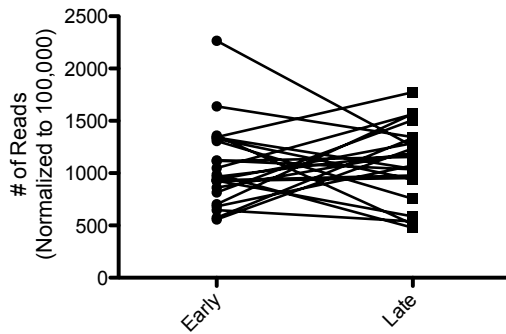
SIV (-) Viridiplantae



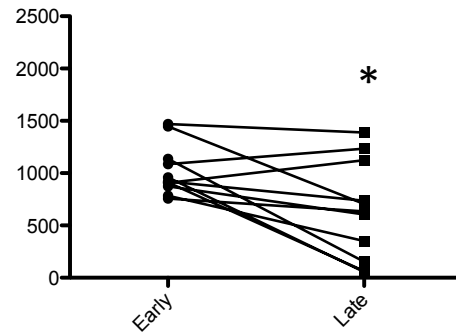
SIV (+) Viridiplantae



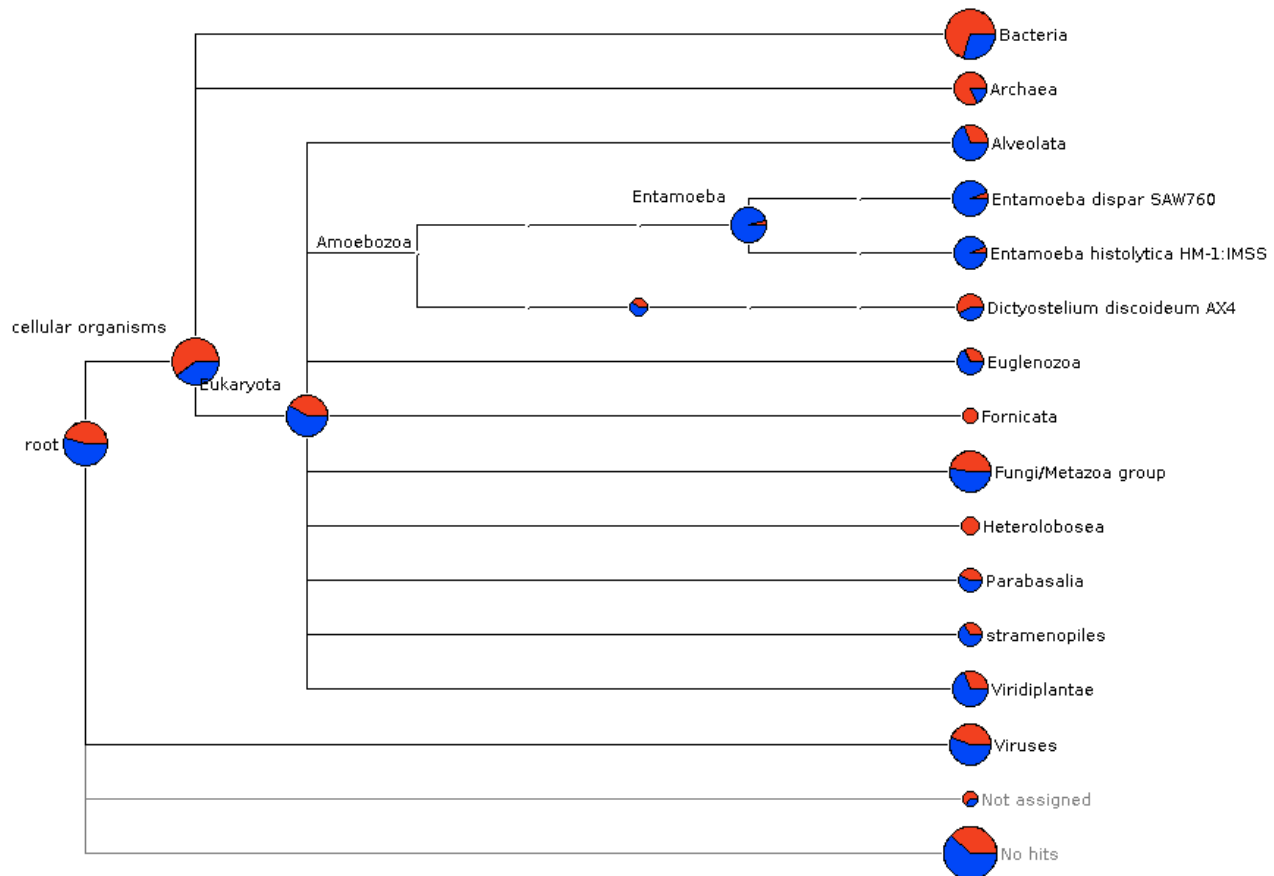
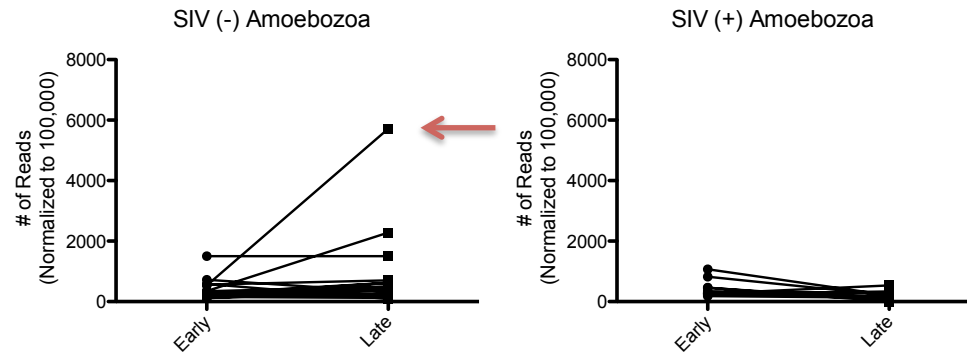
SIV (-) Alveolata



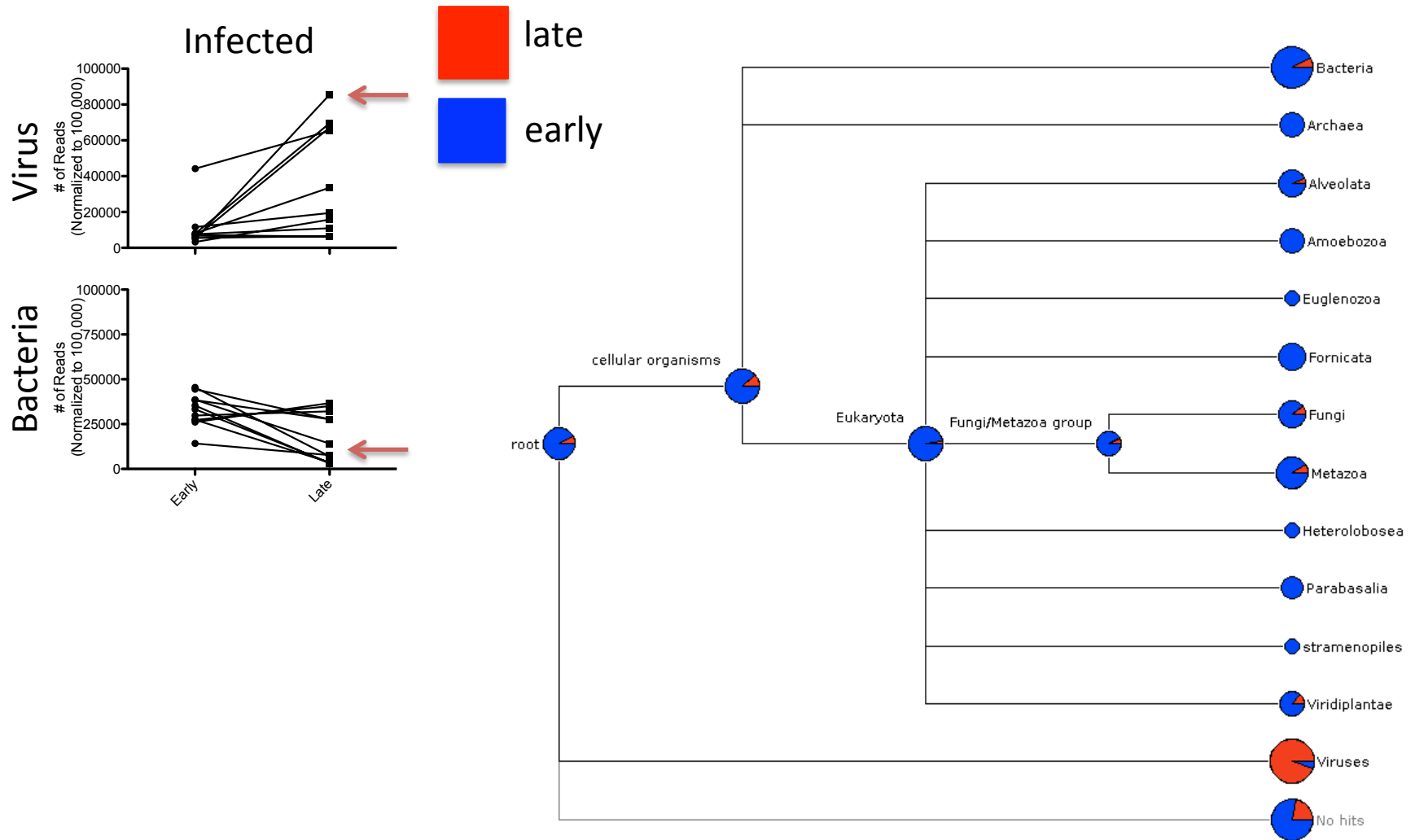
SIV (+) Alveolata



Diagnostic potential

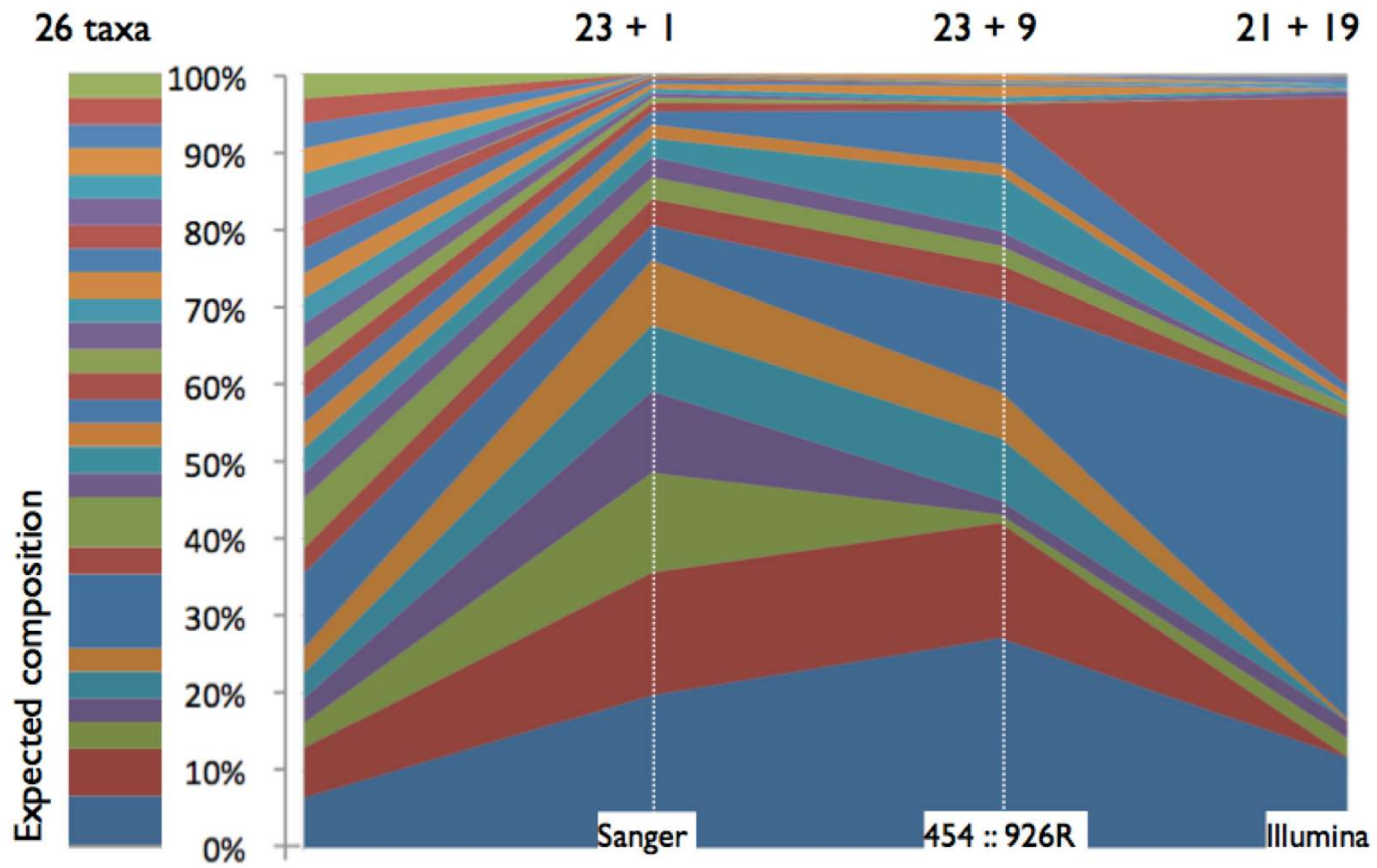


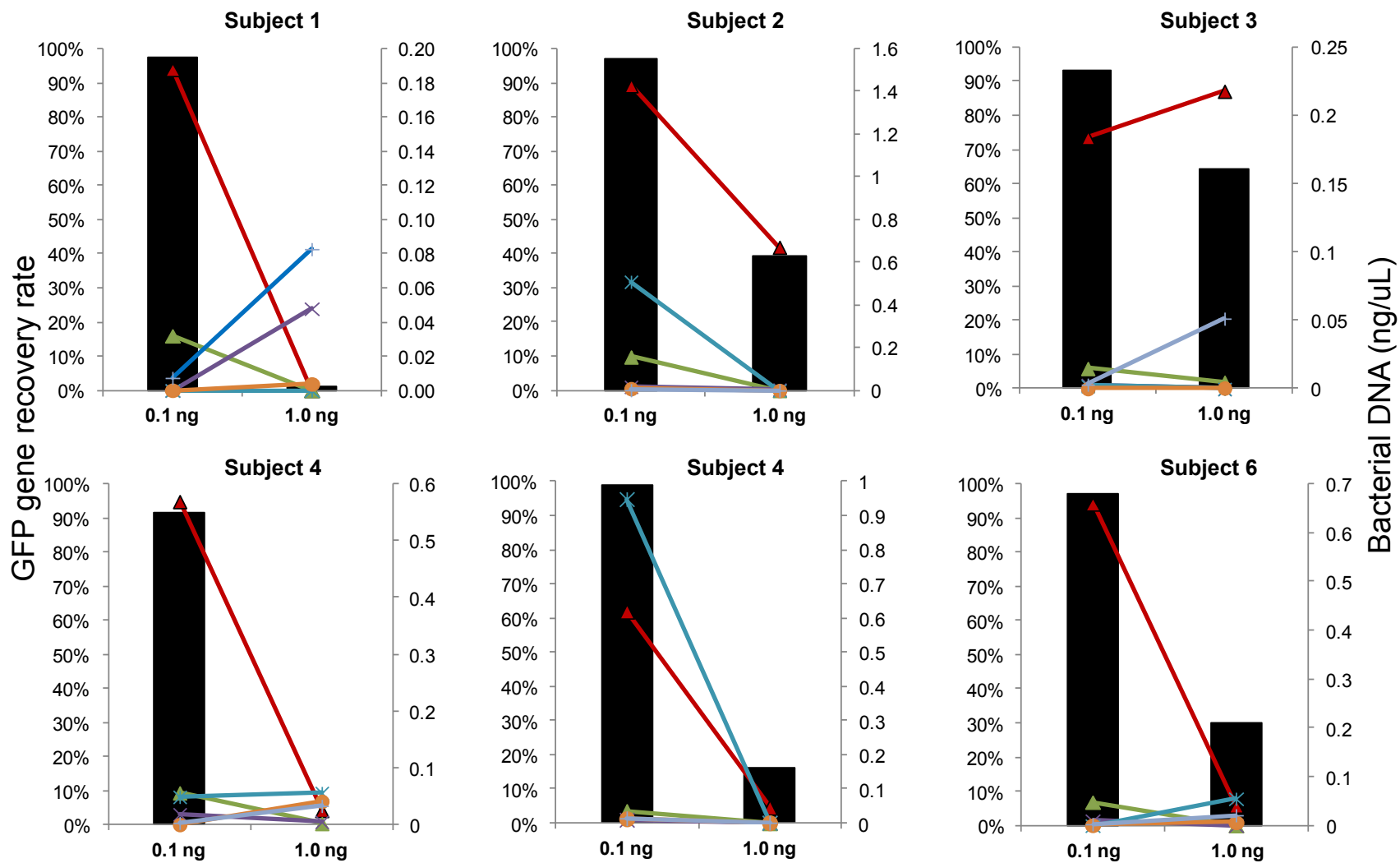
Extreme example





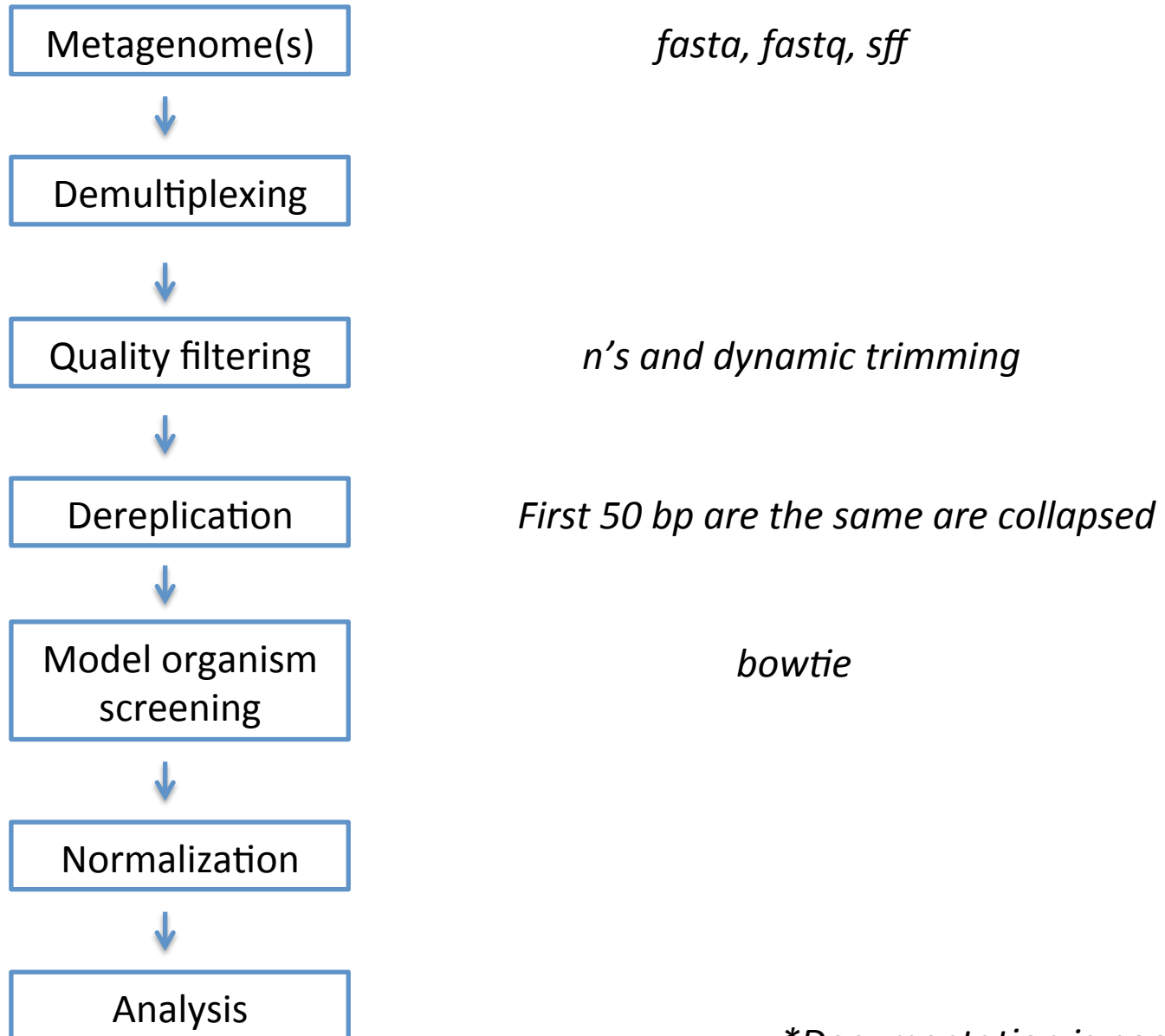
Challenges





mg-RAST

Automated Pipeline*



**Documentation is poor at best*

<http://metagenomics.anl.gov/>

[http://evomics.org/learning/
genomics/metagenomics/mg-rast/](http://evomics.org/learning/genomics/metagenomics/mg-rast/)

