Microbiome Overview

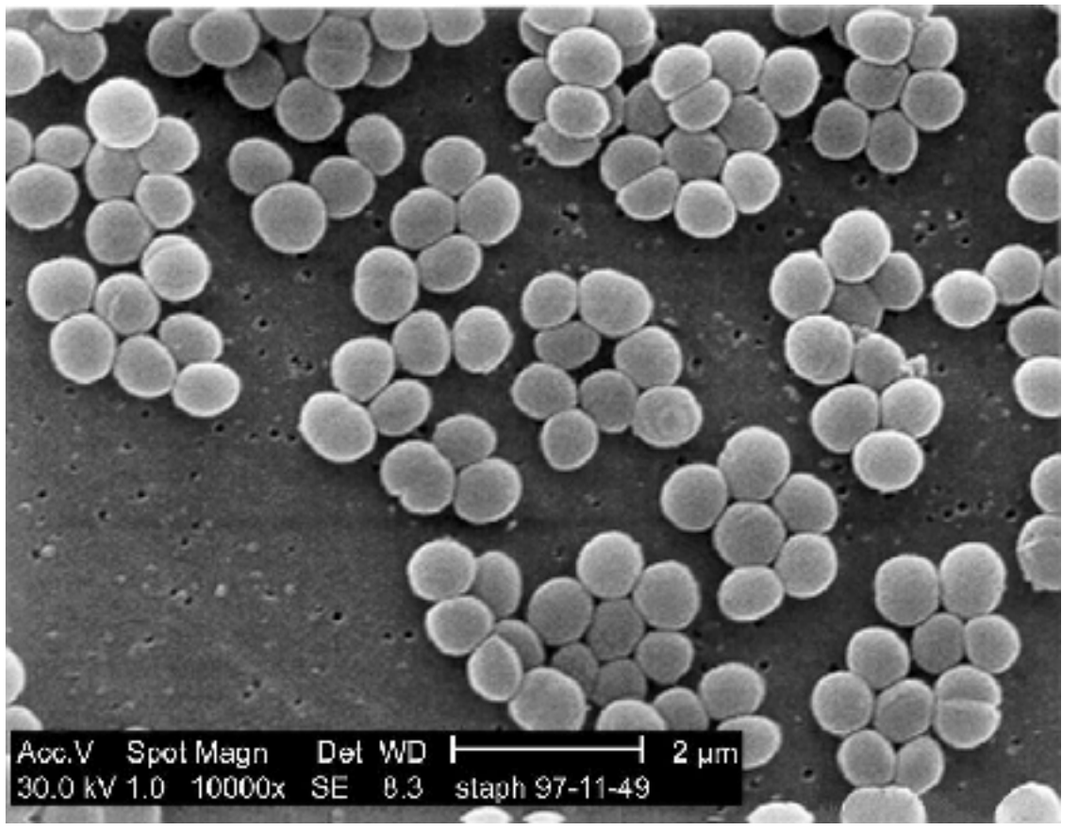
Josh Granek

Microbiome

- The collection of microorganisms (microbes) living in an environment

Microorganism

- microscopic organisms



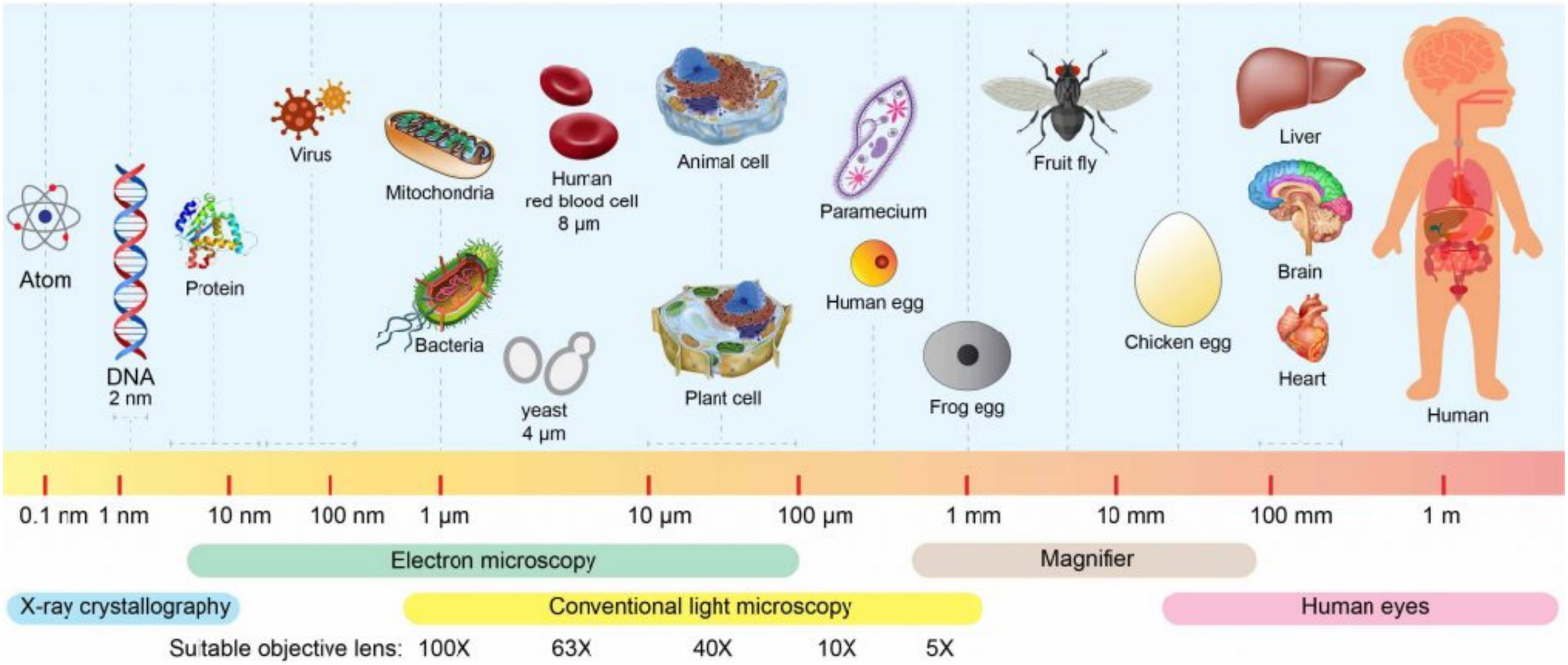
https://en.wikipedia.org/wiki/File:Staphylococcus_aureus_01.jpg

Microbes: Complexity

Humans	Supercomputer	
Fungi*	Tablet	Not as powerful or complex as a supercomputer, but able to do lots of stuff on its own
Bacteria	Cell Phone	Less complicated than Fungi, but still able to do stuff on its own
Viruses	USB drive	Can't do anything on its own, depends on a computer (see: Humans) to do anything

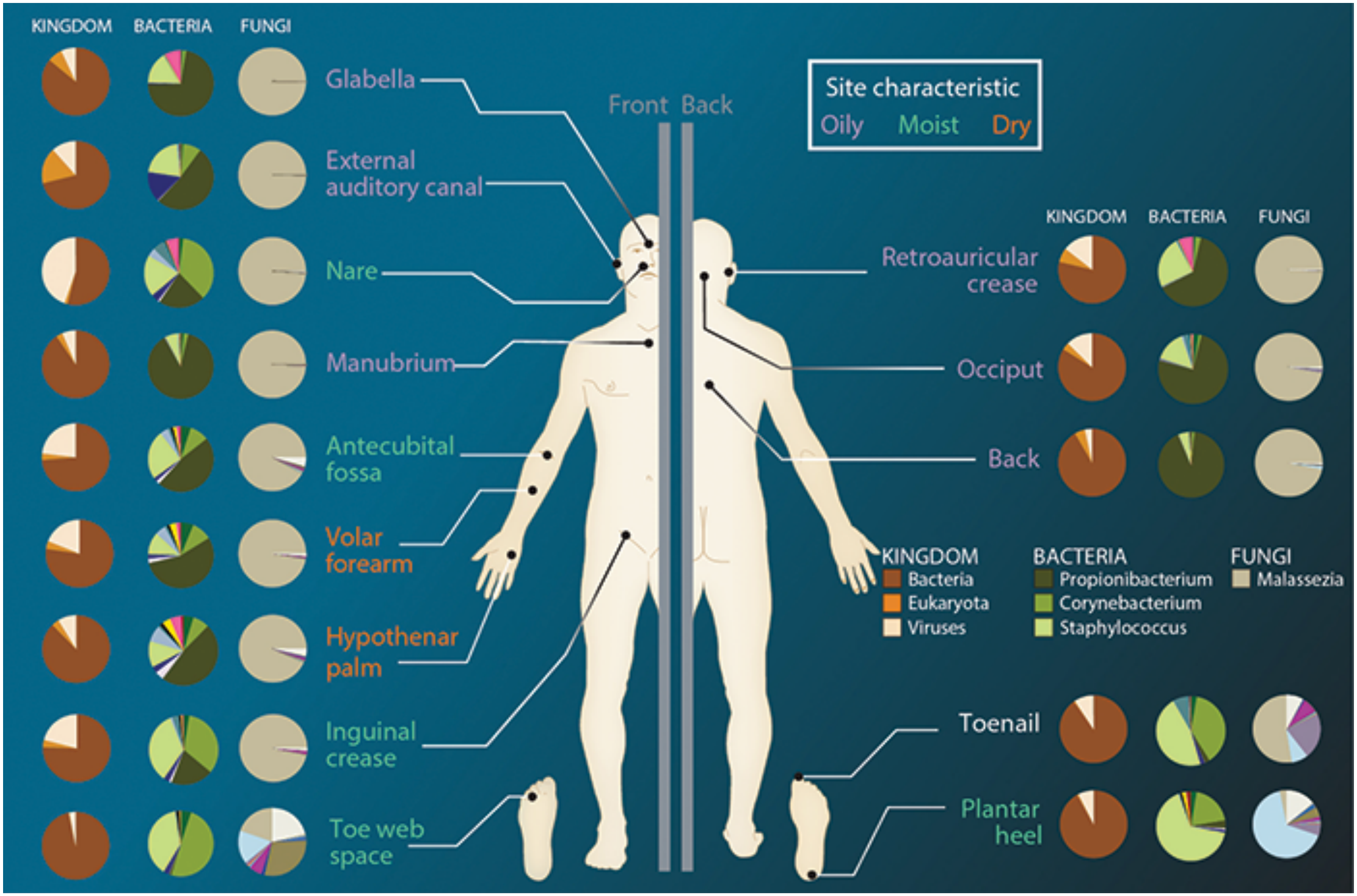
* fungal microbes, there are also multicellular fungi

Microbes: Scale



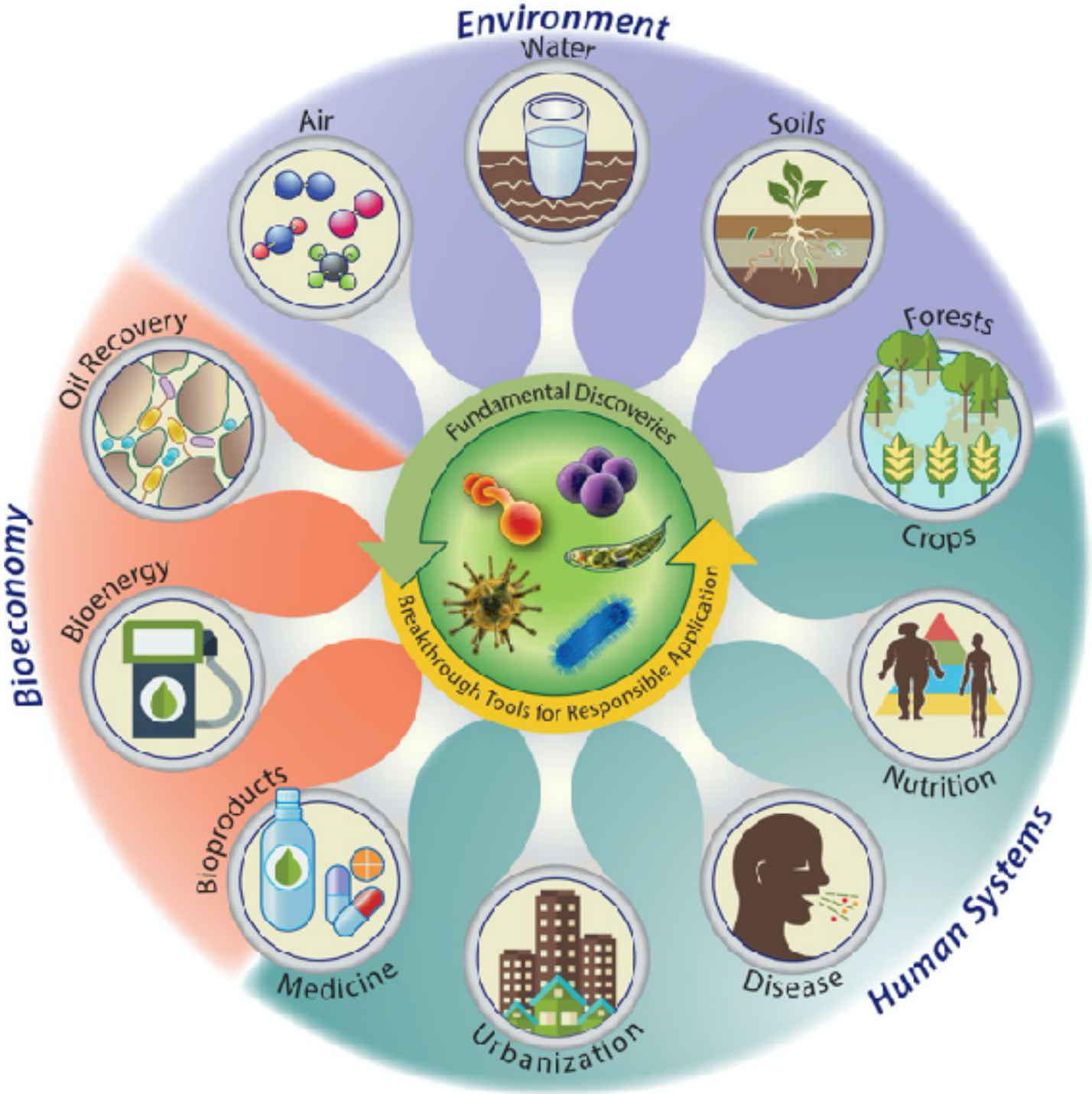
https://rsscience.com/beginners-guide-microscopy/

Microbiomes: Where



https://cen.acs.org/articles/95/i19/Cosmetics-next-microbiome-frontier.html

Microbiomes: Where

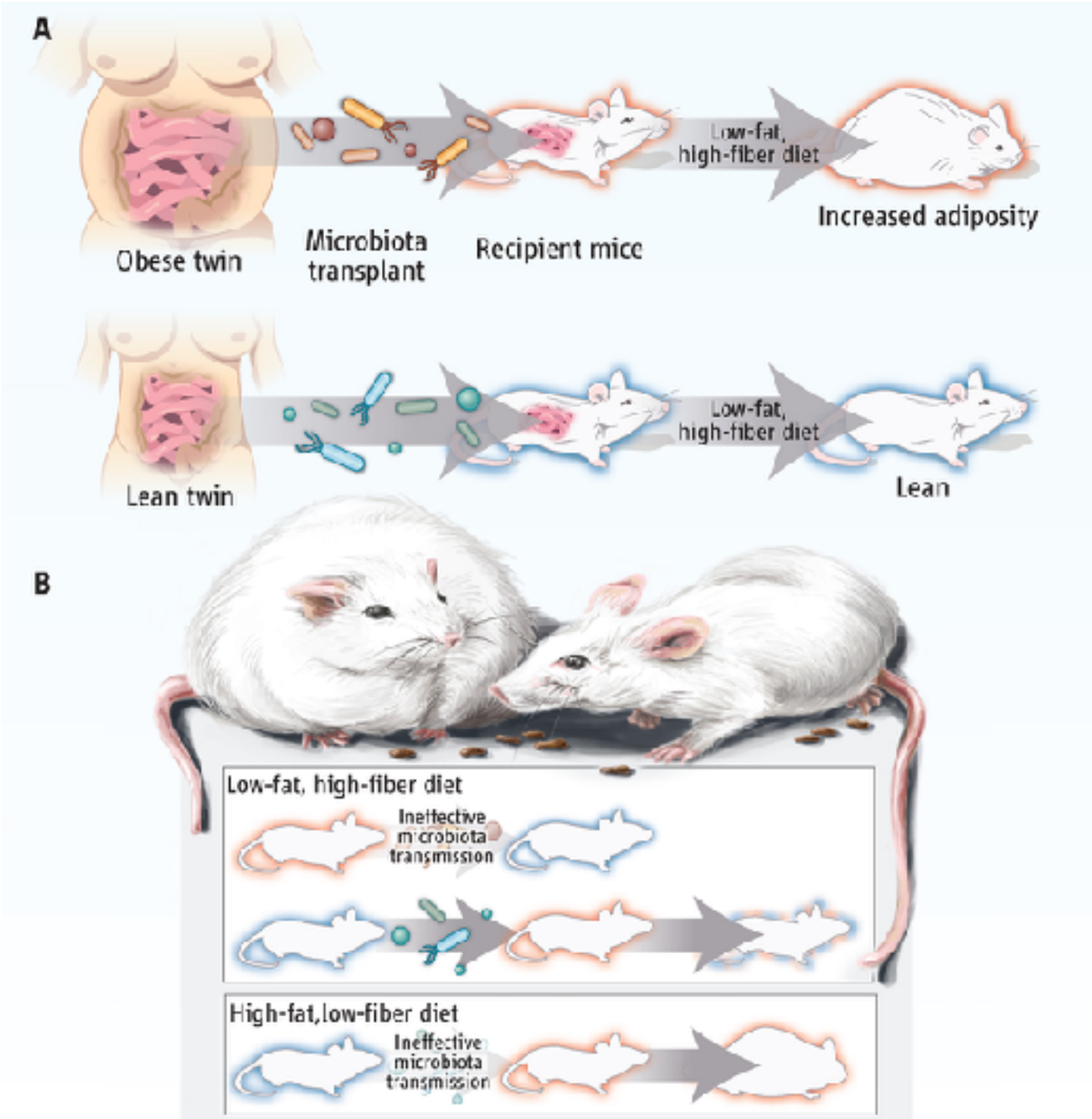


Blaser, et al 2016 https://doi.org/10.1128/mbio.00714-16

Microbiomes in Human Health

- Acne
- Asthma/allergies
- Autism
- Autoimmune diseases
- Cancer
- Dental cavities
- Depression and anxiety
- Diabetes
- Eczema
- Gastric ulcers
- Hardening of the arteries
- Inflammatory bowel diseases
- Malnutrition
- Obesity
- Parkinson's Disease
- Drug Metabolism
- Vaccine Effectiveness
- …

Causation



Walker, et al. Science 2013. https://doi.org/10.1126/science.1243787

Ridaura1, et al. Science 2013. https://doi.org/10.1126/science.1241214

How?

Metagenomics

Metagenomics

Metagenomics

	What
Amplicon	Marker Gene
Shotgun Metagenome	Genomes
Shotgun Metatranscriptome	All RNA

Metagenomics

	What	Information
Amplicon	Marker Gene	Who is Present
Shotgun Metagenome	Genomes	What Genes are Present
Shotgun Metatranscriptome	All RNA	What Genes are Expressed

Metagenomics

	What	Information	Analogy
Amplicon	Marker Gene	Who is Present	Name
Shotgun Metagenome	Genomes	What Genes are Present	CV
Shotgun Metatranscriptome	All RNA	What Genes are Expressed	Twitter Feed

Metagenomics

	What	Information	Analogy	Target Size
Amplicon	Marker Gene	Who is Present	Name	100bp - 1kb
Shotgun Metagenome	Genomes	What Genes are Present	CV	100kb - 100Mb
Shotgun Metatranscriptome	All RNA	What Genes are Expressed	Twitter Feed	100kb - 100Mb

Metagenomics

	What	Information	Analogy	Target Size	Cost
Amplicon	Marker Gene	Who is Present	Name	100bp - 1kb	Low
Shotgun Metagenome	Genomes	What Genes are Present	CV	100kb - 100Mb	High
Shotgun Metatranscriptome	All RNA	What Genes are Expressed	Twitter Feed	100kb - 100Mb	High

Metagenomics

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	What	Information	Analogy	Target Size	Cost
Amplicon	Marker Gene	Who is Present	Name	100bp - 1kb	Low
Shotgun Metagenome	Genomes	What Genes are Present	CV	100kb - 100Mb	High
Shotgun Metatranscriptome	All RNA	What Genes are Expressed	Twitter Feed	100kb - 100Mb	High

Amplicon Sequencing

PCR amplify and sequence a marker gene

Amplicon Sequencing

PCR amplify and sequence a marker gene

	Marker Gene
Bacteria	16S rRNA
Fungi	18S or ITS rRNA
Archaea	16S rRNA
Protozoa	18S rRNA
Viruses	?????

A Whale of an Analogy

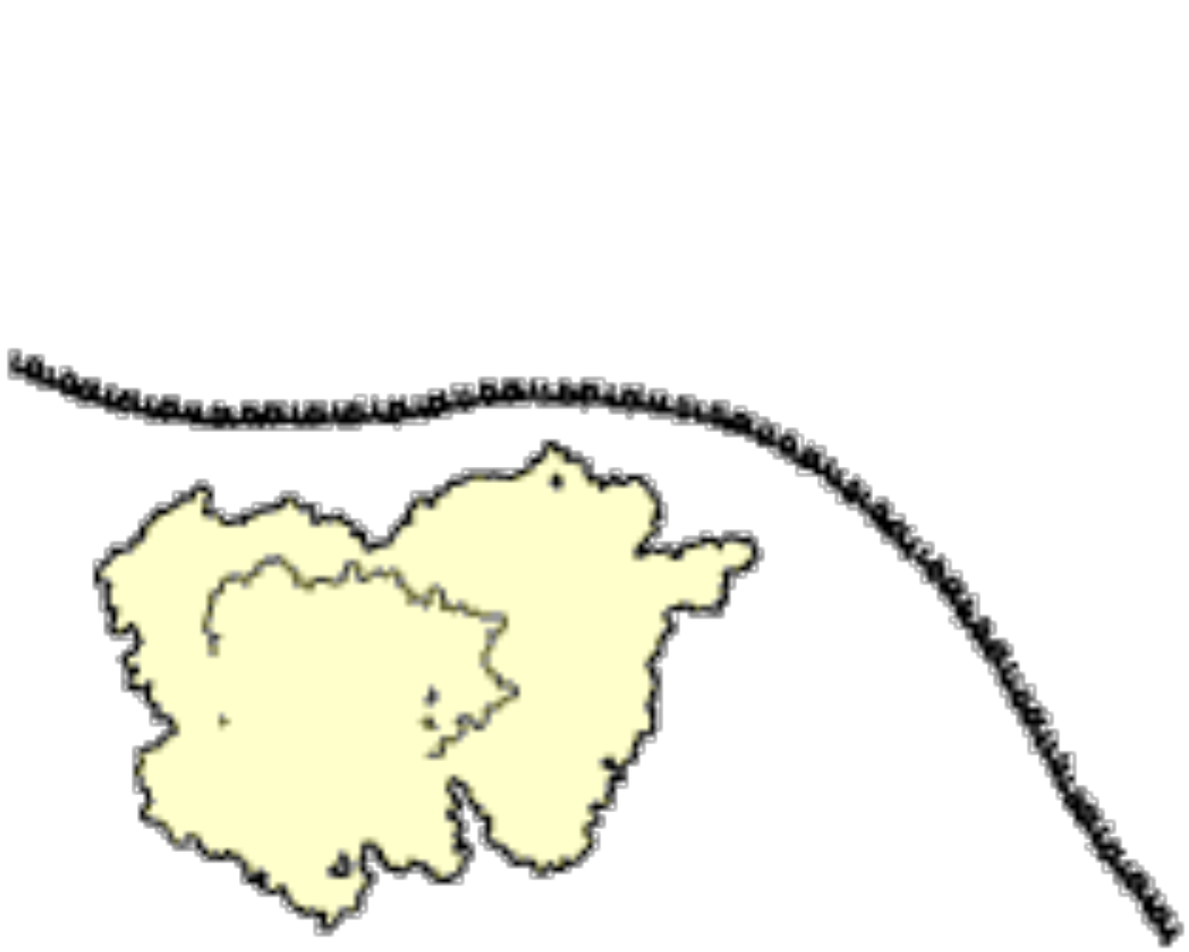
- Amplicon: 250 characters ("These reflections just here are occasioned by the circumstance that after we were all seated at the table, and I was preparing to hear some good stories about whaling; to my no small surprise, nearly every man maintained a profound silence. And not o")
- Whole book: 1.2×10^6 characters

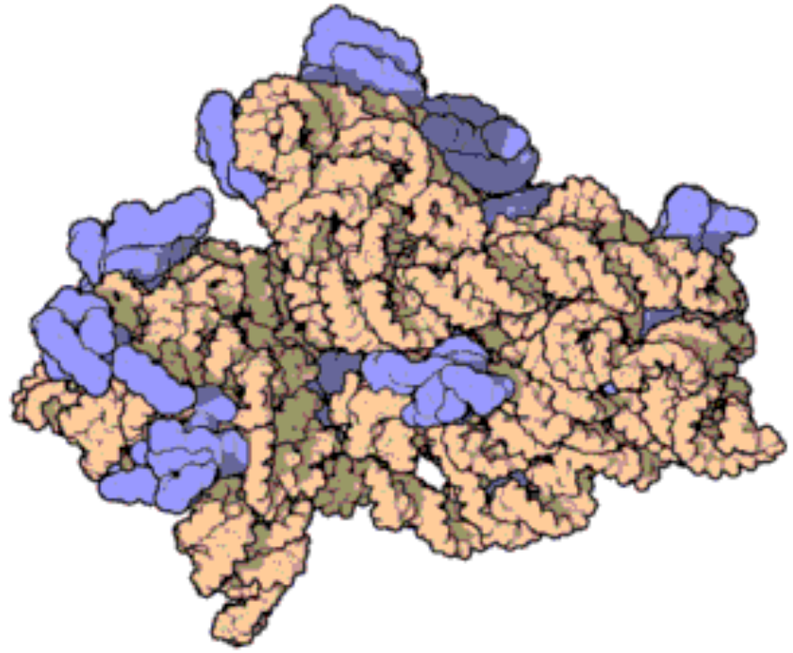
Metagenomics

	What	Information	Analogy	Target Size	Cost	Discovery?
Amplicon	Marker Gene	Who is Present	Name	100bp - 1kb	Low	+/-
Shotgun Metagenome	Genomes	What Genes are Present	CV	100kb - 100Mb	High	++
Shotgun Metatranscriptome	All RNA	What Genes are Expressed	Twitter Feed	100kb - 100Mb	High	++

The 16S Amplicon

The Ribosome





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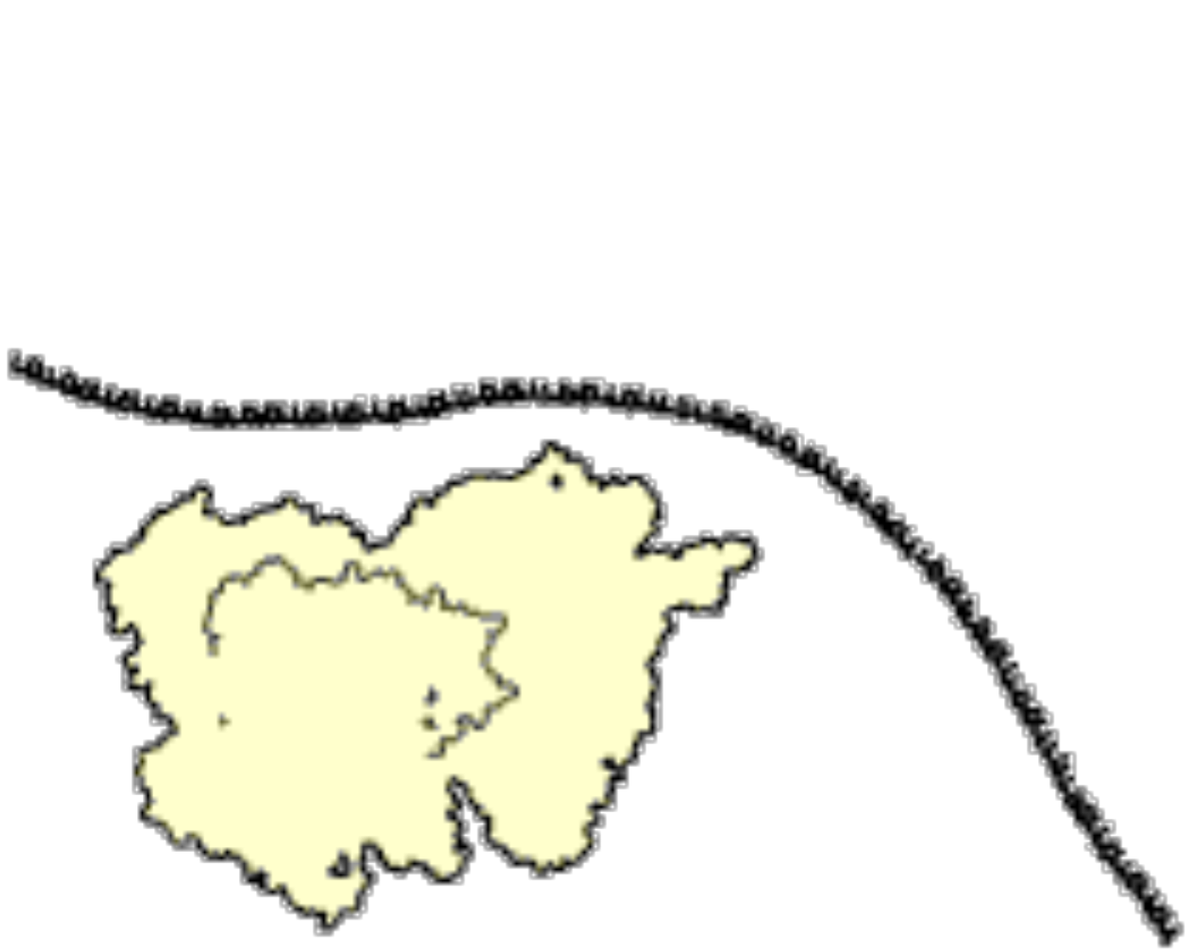
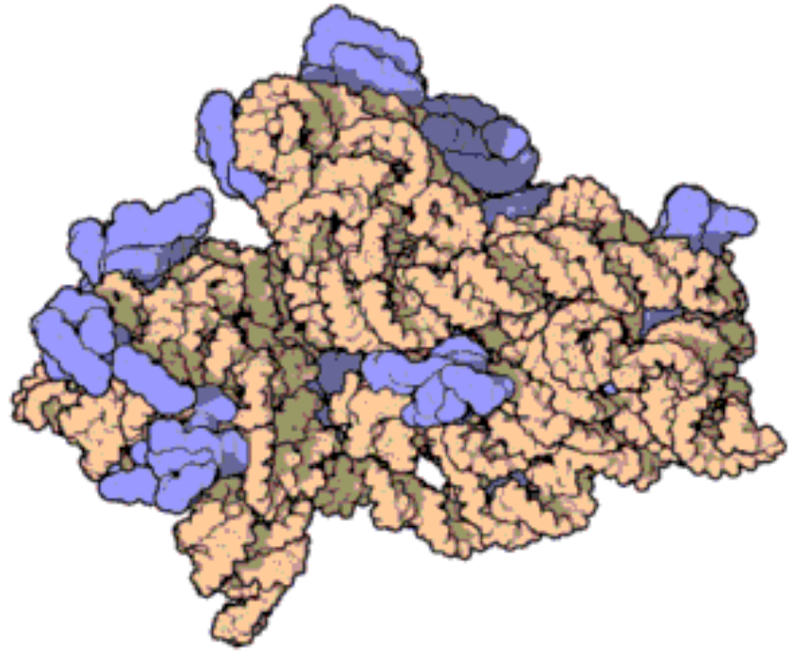
The Ribosome





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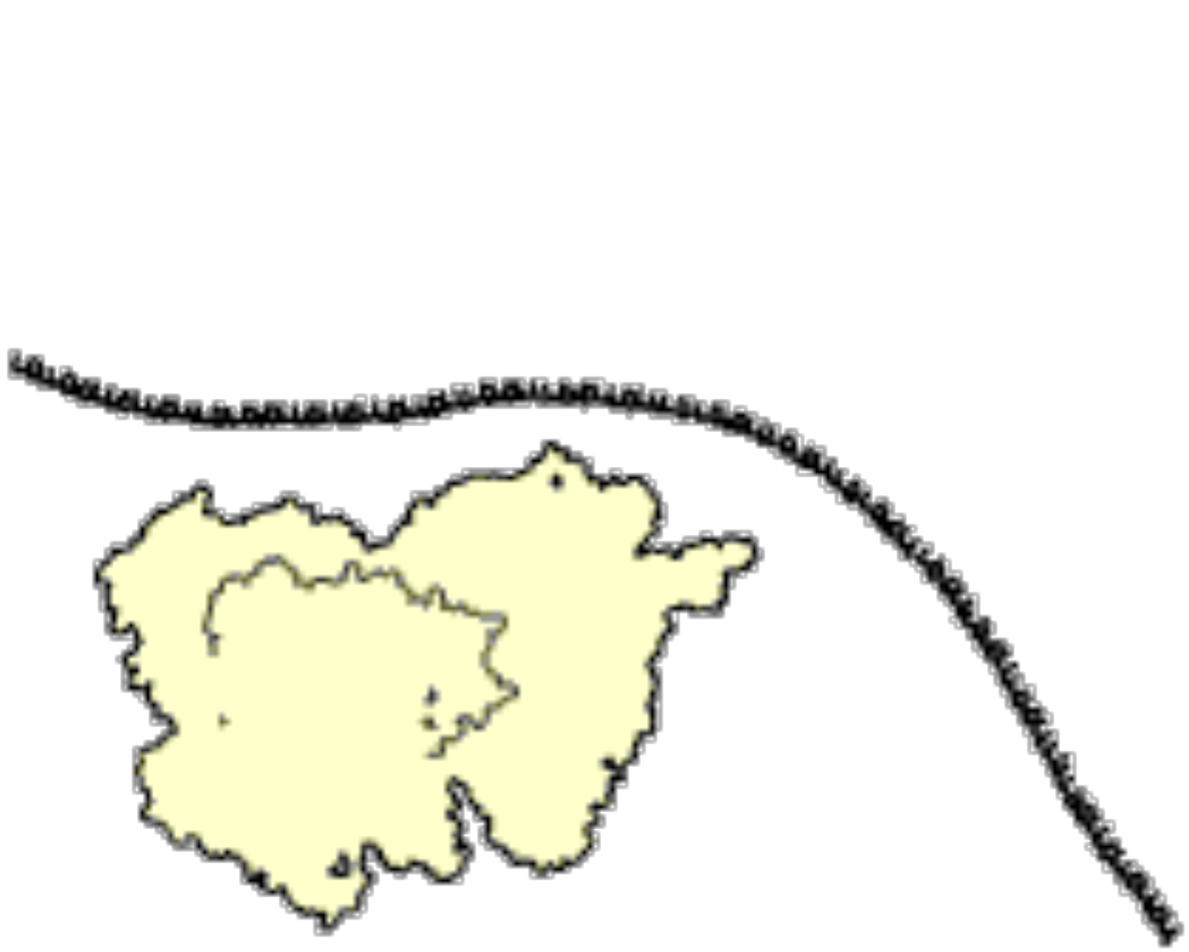

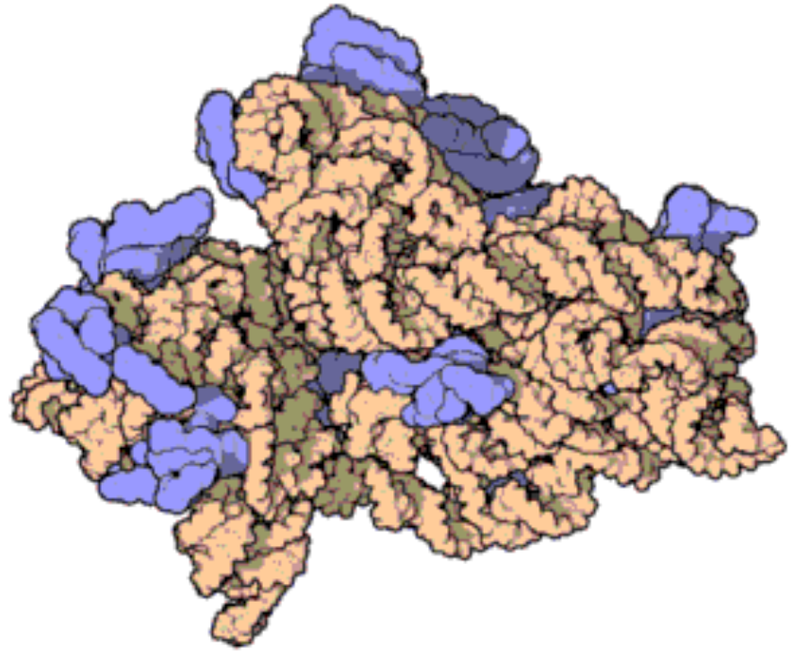

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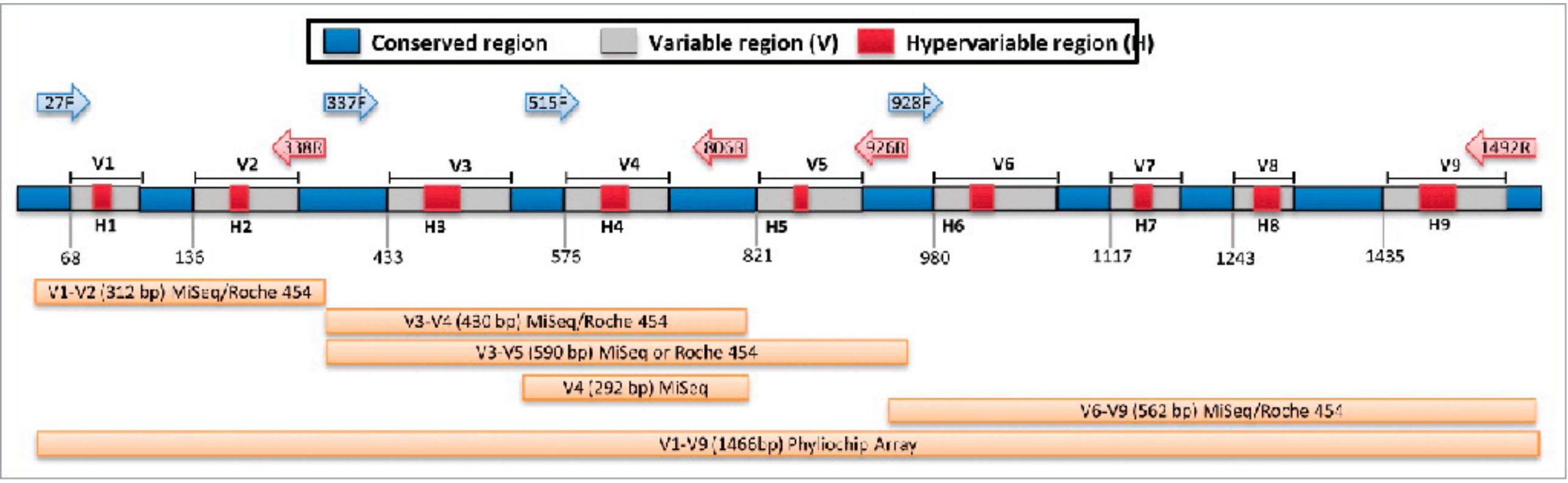
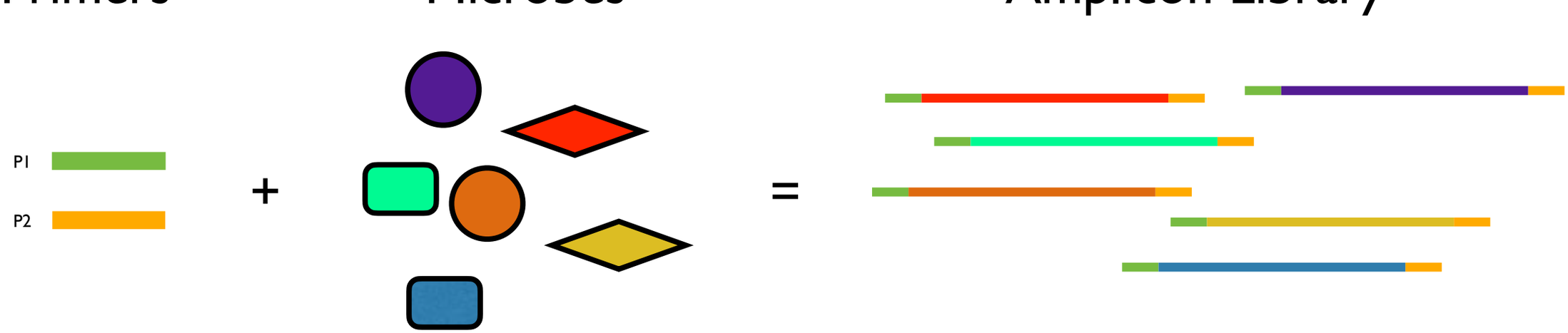
16S rRNA Gene



Shahi, et al. Gut Microbes. 2017. https://doi.org/10.1080/19490976.2017.1349041

Sequencing Details

Amplicon Sequencing

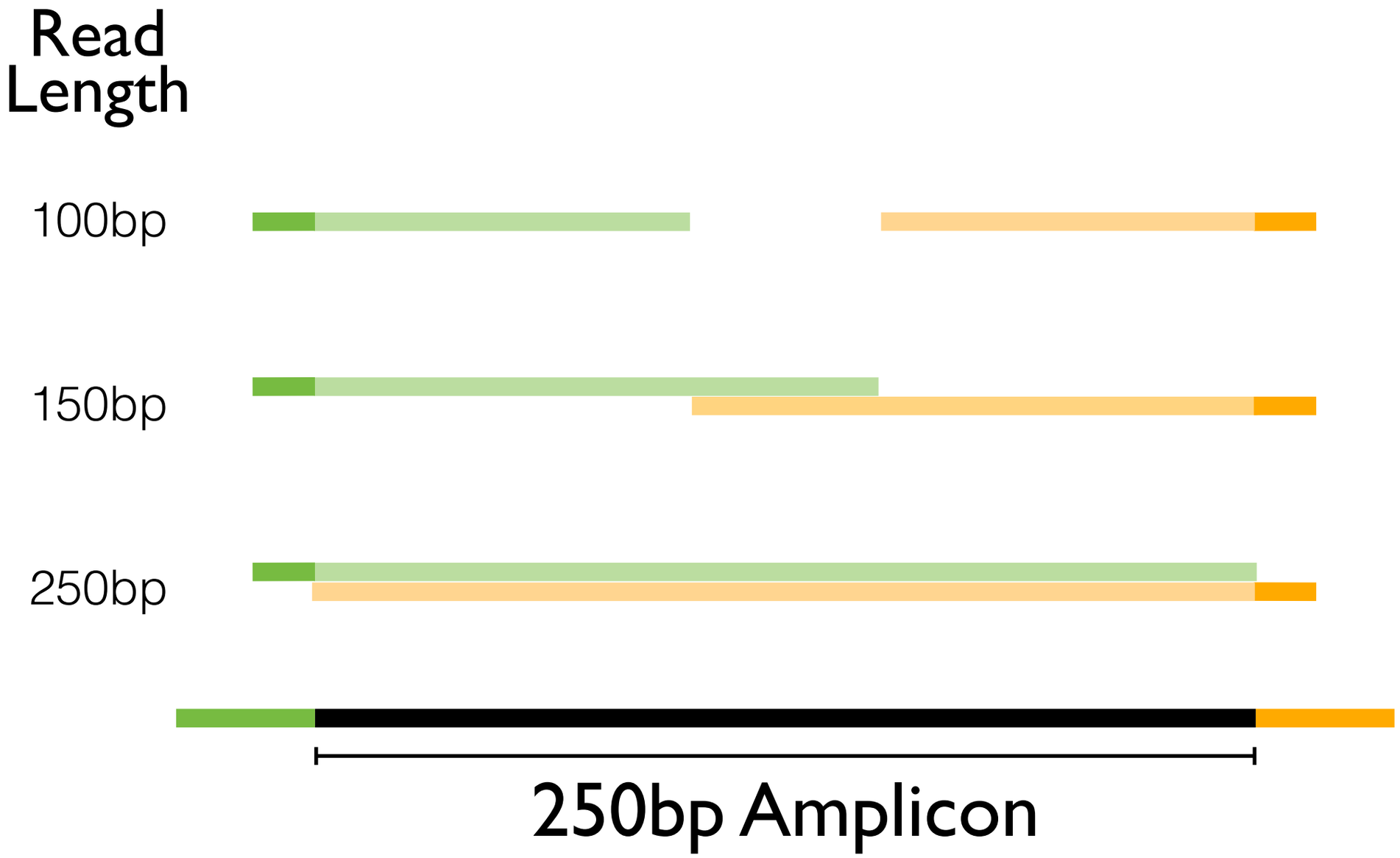


Sequencing Library

Amplicon Library

Shotgun Library

Read Length

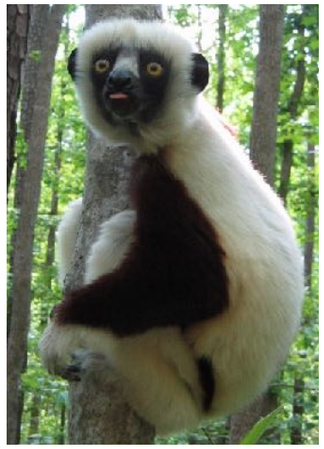


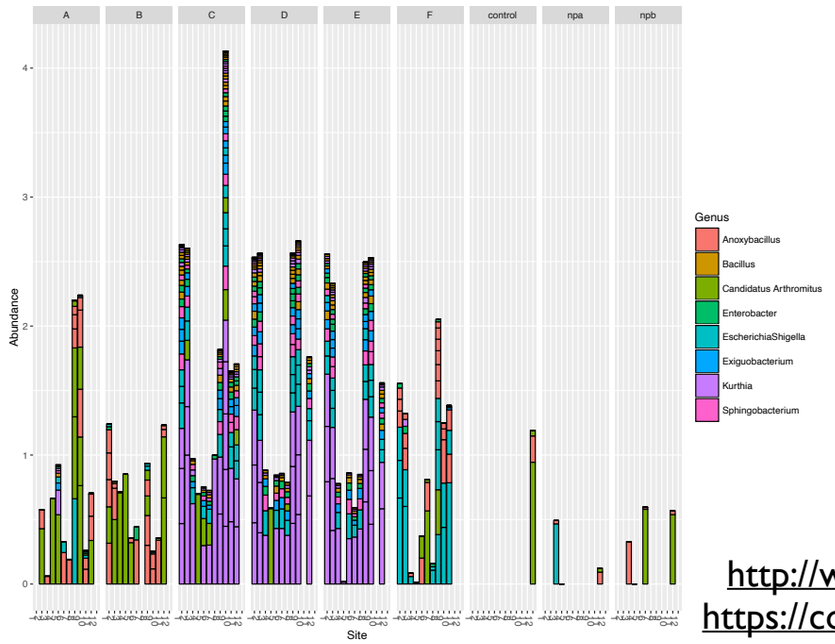
Amplicon Analysis

Big Picture

1. What is present?
2. How much?
3. Are there differences between treatments, host species, …?
4. What are the differences?

Molecular Biology





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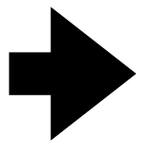
Bioinformatic Analysis



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+
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@M00698:36:000000000-AFBEL:1:1101:16483:1412 1:N:0:0
CTGCCAGTTGAACGACGGCGAGCAGTTATAAGCCAGCAGTTTGCCCGGATATTTCGCGTGGATAGCTTGTGCAAAGCGACGCGCCAGTTCCAGATCCGGCG
+
AAABBFFFFFFFGGGGGGGGGGGGHHHHHHHHHGHGHGHHHHHGHHHGGGGGHHHHGGGGGGGHHHGHHFFHHHHHGHGGGGGGGGGGHHHHHHHHHHHGGG
@M00698:36:000000000-AFBEL:1:1101:15928:1413 1:N:0:0
GTAAAGTCCTGAGTGATACCGGCAACTTTTACCCCCAGTCCCACTTTCGAACCGGCAAACATATCGGCAAAAGAGGCCGTGCCTGATTTAAAGCCGTAGGT
+
```

	Sample 1	Sample 2	…	Sample N
Bacteria 1	1	10		
Bacteria 2	100	0		
…				
Bacteria N				

https://commons.wikimedia.org/wiki/File:Illumina_MiSeq_sequencer.jpg

Statistical Analysis

	Sample 1	Sample 2	…	Sample N
Bacteria 1				
Bacteria 2				
…				
Bacteria N				

1. What is present?
2. How much?
3. Are there differences between treatments, host species, …?
4. What are the differences?

Caveat

http://www.lebleu.com

The End