Microbiome Overview

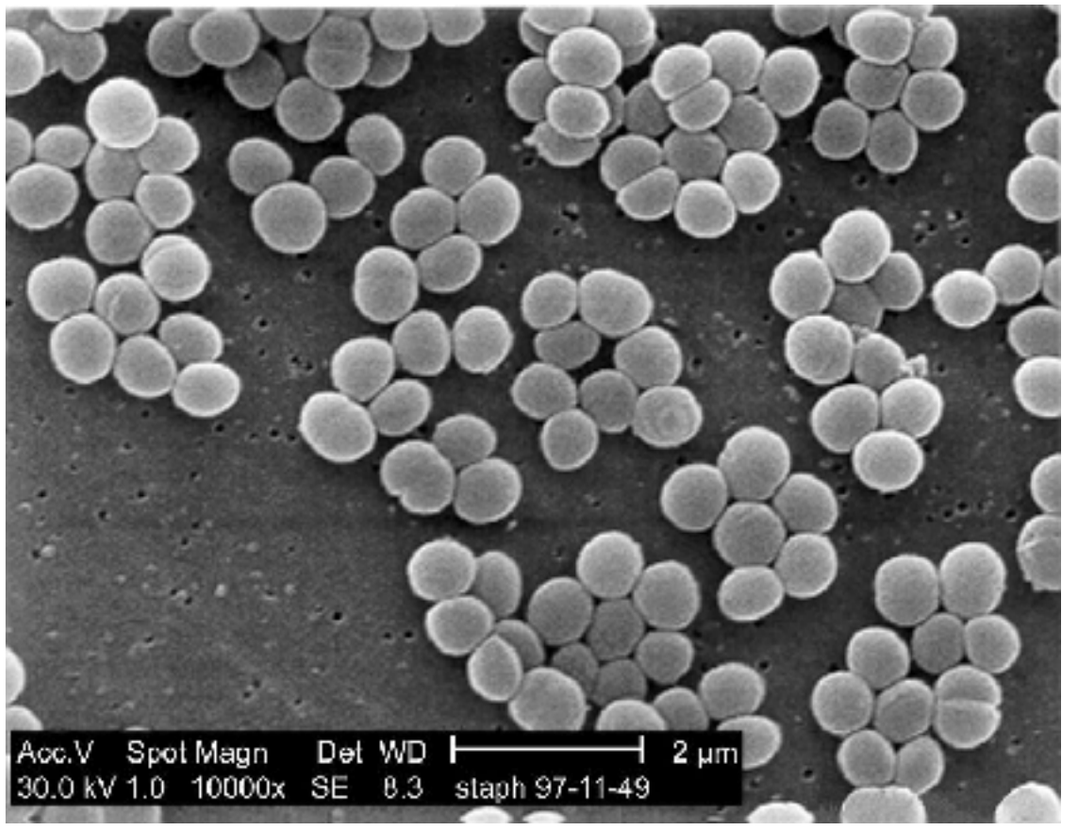
Josh Granek

Microbiome

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

Microorganism

- microscopic organisms



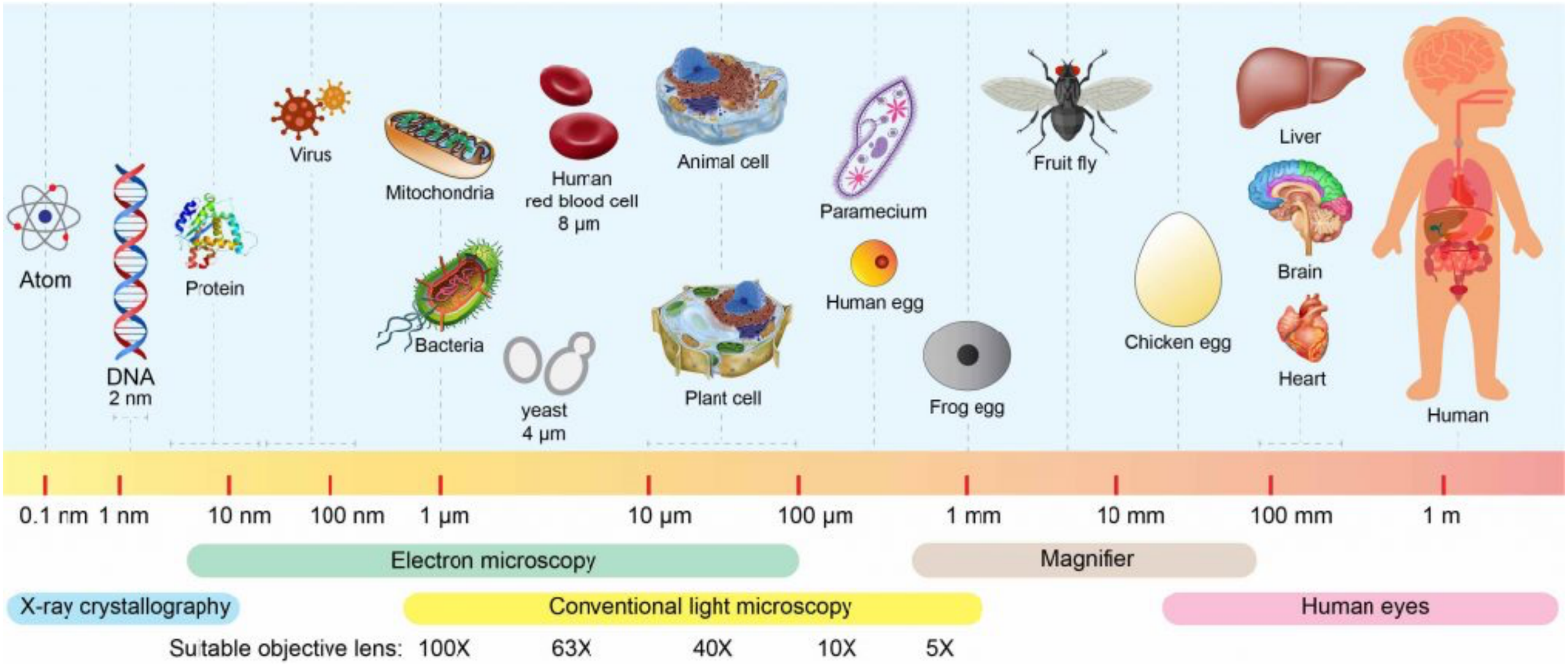
https://en.wikipedia.org/wiki/File:Staphylococcus_aureus_01.jpg

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

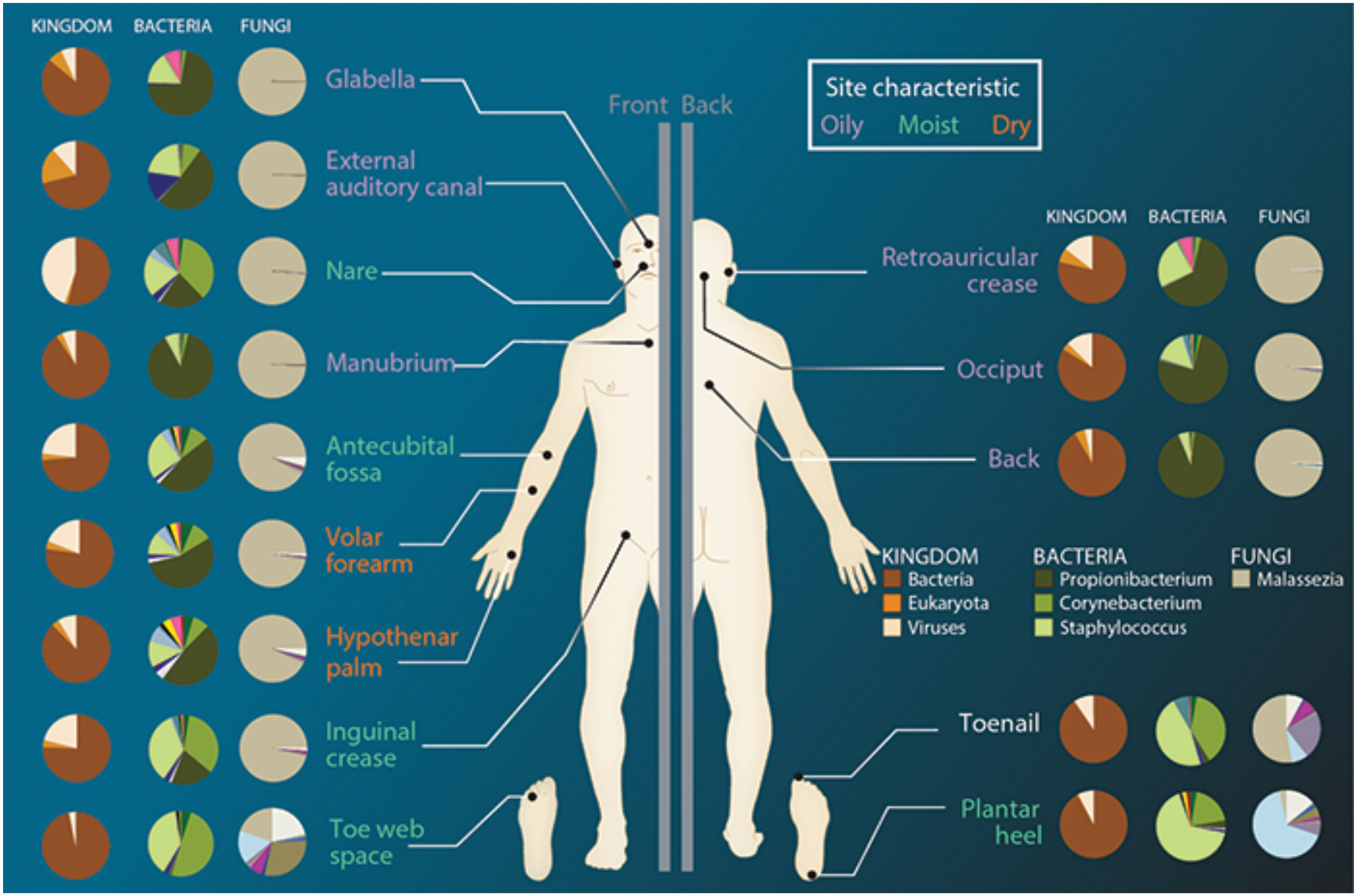
* fungal microbes, there are also multicellular fungi

Microbes: Scale



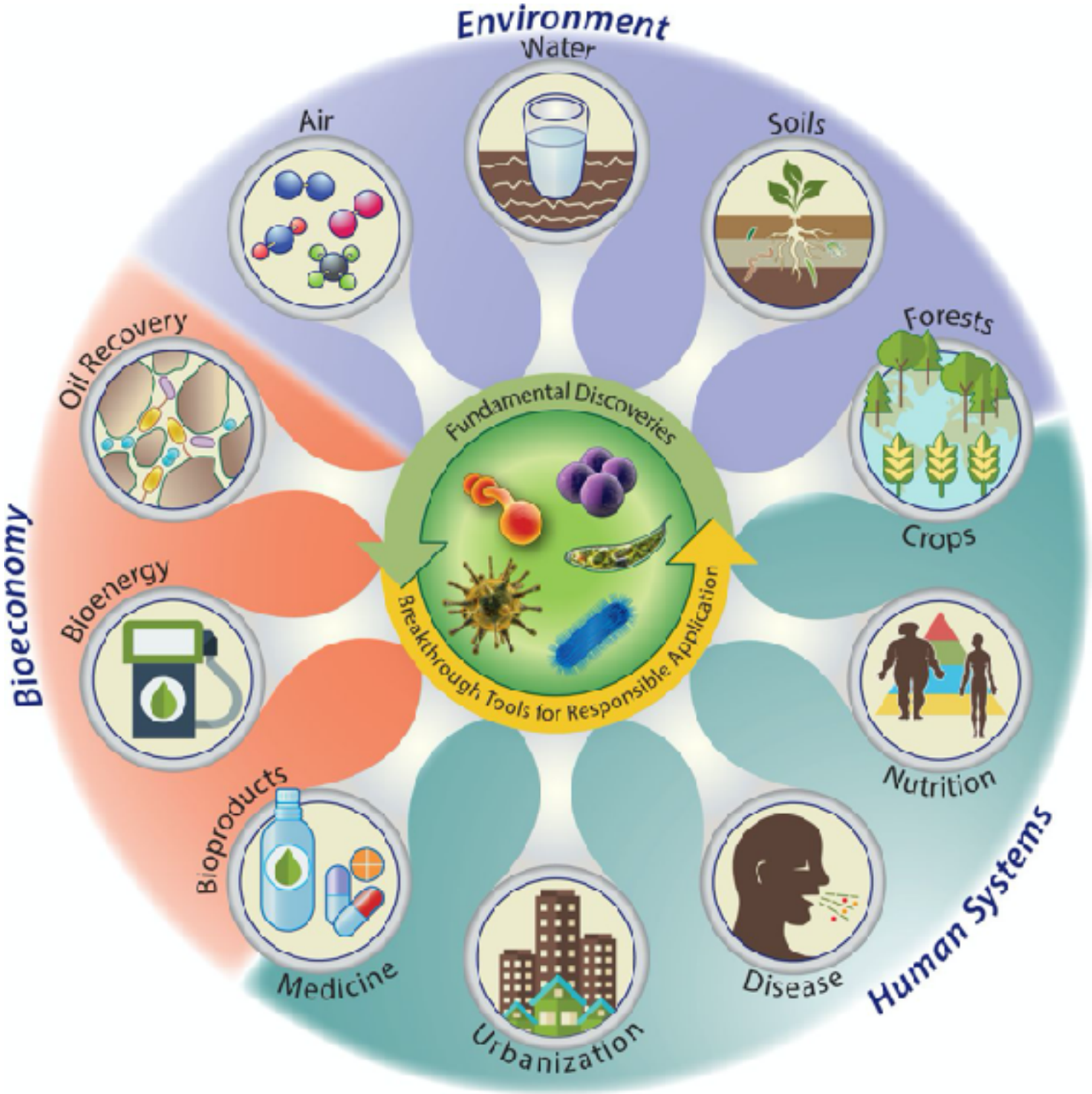
https://rsscience.com/beginners-guide-microscopy/

Microbiomes: Where



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

Microbiomes: Where

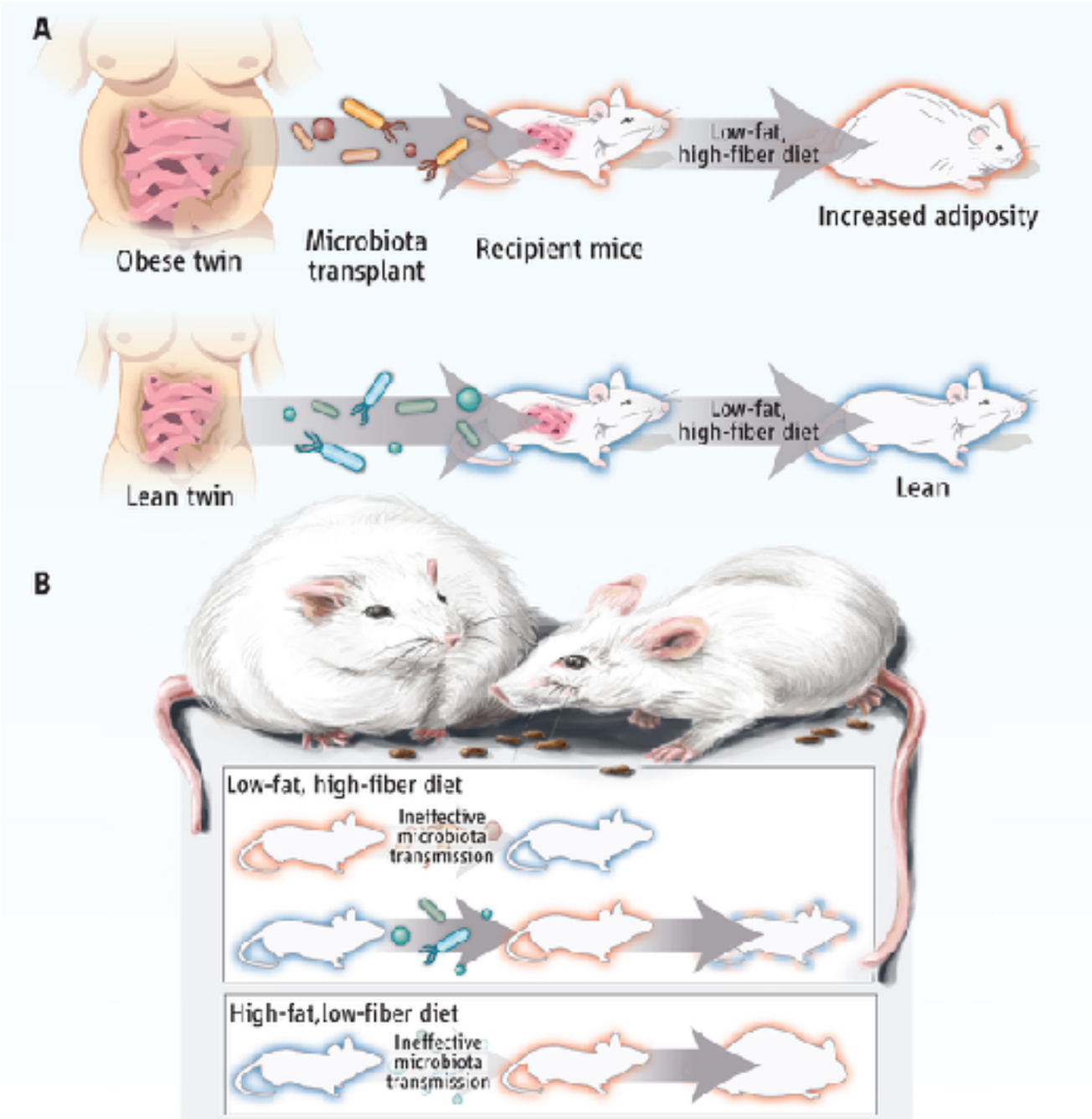


Blaser, et al 2016 https://doi.org/10.1128/mbio.00714-16

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

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

Fungal genome and mating system transitions facilitated by chromosomal translocations involving intercentromeric recombination.
Sun S, Yadav V, Billmyre RB, Cuomo CA, Nowrousian M, Wang L, Souciet JL, Boekhout T, Porcel B, Wincker P, **Granek JA**, Sanyal K, Heitman J.
PLoS Biol. 2017 Aug 11;15(8):e2002527. doi: 10.1371/journal.pbio.2002527. eCollection 2017 Aug.
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2.
Elucidation of the calcineurin-Crz1 stress response transcriptional network in the human fungal pathogen Cryptococcus neoformans.
Chow EW, Clancey SA, Billmyre RB, Averette AF, **Granek JA**, Mieczkowski P, Cardenas ME, Heitman J.
PLoS Genet. 2017 Apr 4;13(4):e1006667. doi: 10.1371/journal.pgen.1006667. eCollection 2017 Apr.
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3.
Evidence for distinct brain networks in the control of rule-based motor behavior.
Granek JA, Sergio LE.
J Neurophysiol. 2015 Aug;114(2):1298-309. doi: 10.1152/jn.00233.2014. Epub 2015 Jul 1.
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4.
Rapid mapping of insertional mutations to probe cell wall regulation in Cryptococcus neoformans.
Esher SK, **Granek JA**, Alspaugh JA.
Fungal Genet Biol. 2015 Sep;82:9-21. doi: 10.1016/j.fgb.2015.06.003. Epub 2015 Jun 23.
PMID: 26112692 **Free PMC Article**
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5.
Integrating chemical mutagenesis and whole-genome sequencing as a platform for forward and reverse genetic analysis of Chlamydia.
Kokes M, Dunn JD, **Granek JA**, Nguyen BD, Barker JR, Valdivia RH, Bastidas RJ.
Cell Host Microbe. 2015 May 13;17(5):716-25. doi: 10.1016/j.chom.2015.03.014. Epub 2015 Apr 23.
PMID: 25920978 **Free PMC Article**
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6.
Antifungal drug resistance evoked via RNAi-dependent epimutations.
Calo S, Shertz-Wall C, Lee SC, Bastidas RJ, Nicolás FE, **Granek JA**, Mieczkowski P, Torres-Martínez S, Ruiz-Vázquez RM, Cardenas ME, Heitman J.
Nature. 2014 Sep 25;513(7519):555-8. doi: 10.1038/nature13575. Epub 2014 Jul 27.
PMID: 25079329 **Free PMC Article**
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7.
Decoupled visually-guided reaching in optic ataxia: differences in motor control between canonical and non-canonical orientations in space.
Granek JA, Pisella L, Stemberger J, Vighetto A, Rossetti Y, Sergio LE.
PLoS One. 2013 Dec 31;8(12):e86138. doi: 10.1371/journal.pone.0086138. eCollection 2013.
PMID: 24392035 **Free PMC Article**
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8.

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	?????

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

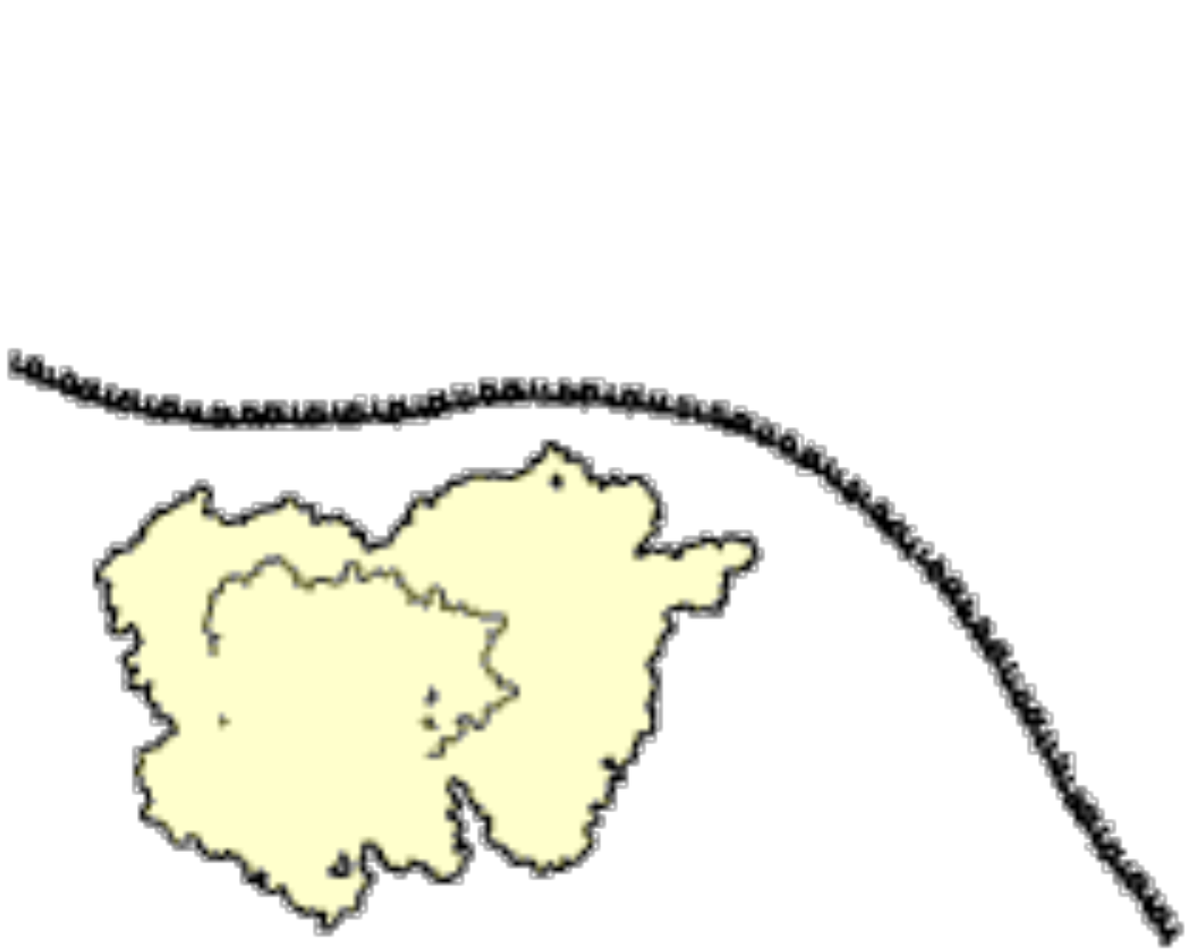
	What	Information	Analogy	Target Size	Cost	Discovery?
Amplicon	Marker Gene	Who is Present	Name	100bp - 1kb	Low	+/-
Shotgun Metagenome	Genomes	What Genes are Present	CV	100kb - 100Mb	High	++
Shotgun Metatranscriptome	All RNA	What Genes are Expressed	Twitter Feed	100kb - 100Mb	High	++

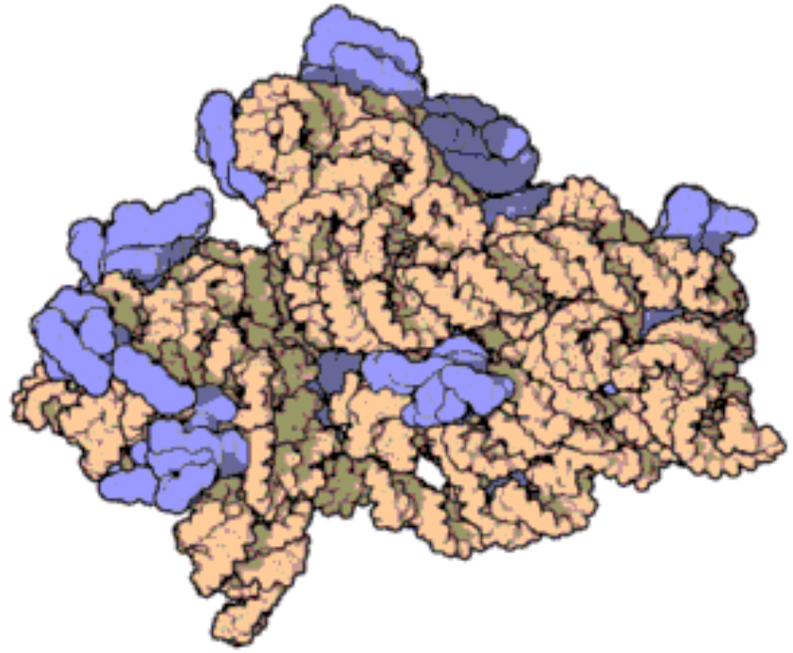
A Whale of an Analogy

- 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

The 16S Amplicon

The Ribosome

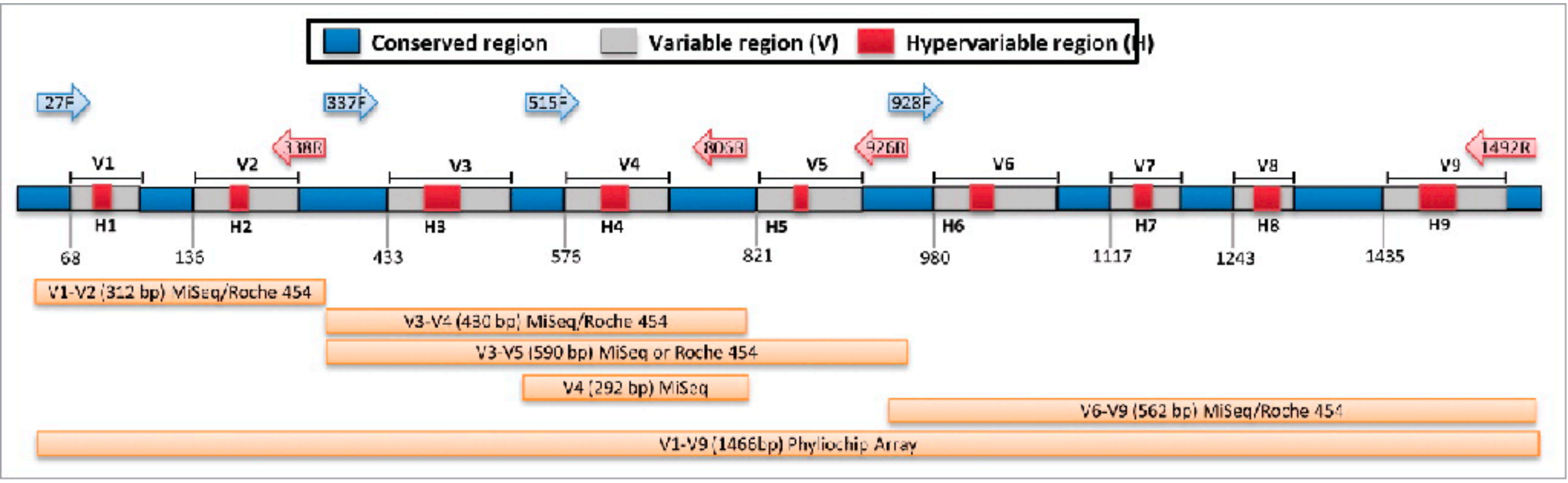




https://upload.wikimedia.org/wikipedia/commons/9/94/Protein_translation.gif

https://upload.wikimedia.org/wikipedia/commons/9/90/010_small_subunit-1FKA.gif

16S rRNA Gene



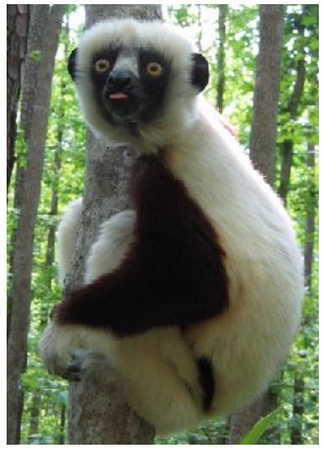
Shahi, et al. Gut Microbes. 2017. https://doi.org/10.1080/19490976.2017.1349041

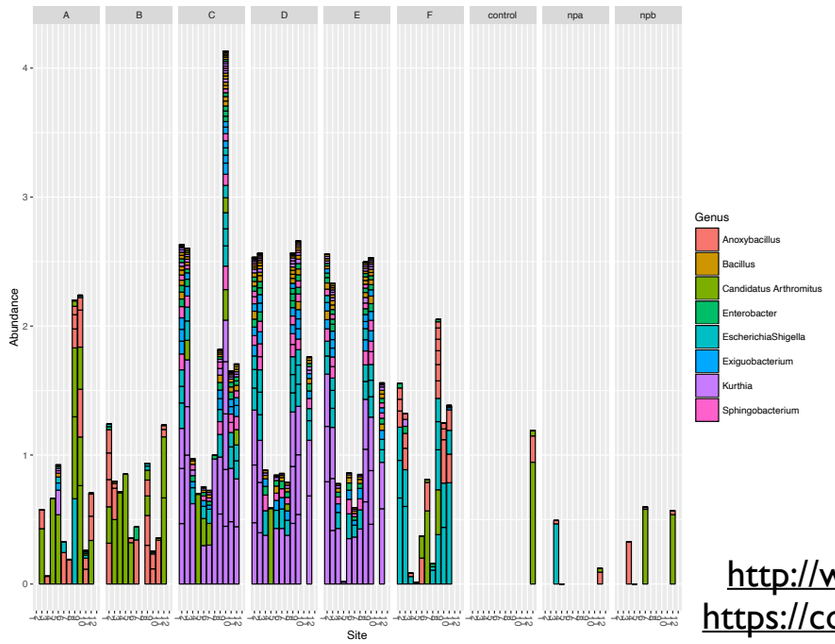
Amplicon Analysis

Big Picture

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

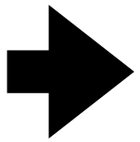
Molecular Biology





http://www.geograph.org.uk/photo/2847164
https://commons.wikimedia.org/wiki/File:Pcr.jpg
https://commons.wikimedia.org/wiki/File:Illumina_MiSeq_sequencer.jpg

Bioinformatic Analysis



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+
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+
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+
```

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