Introduction to metabarcoding

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Which is our question?

- Who is in there?
- What can they do?
- What are they doing?

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Molecular barcoding

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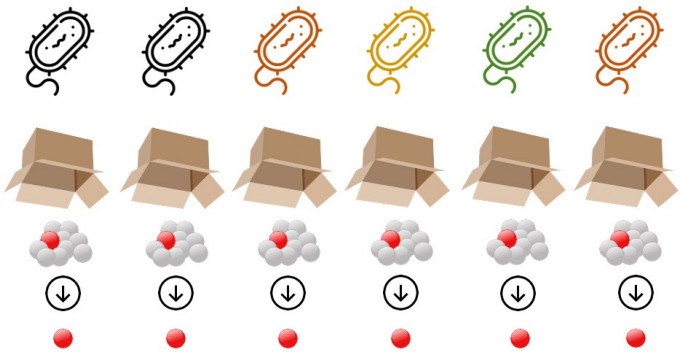
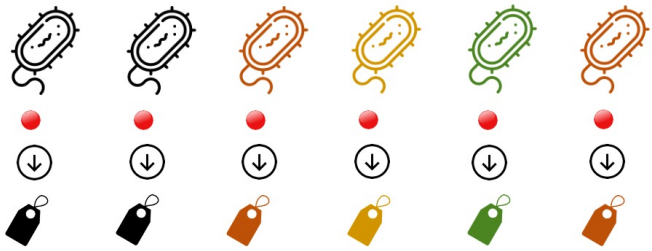
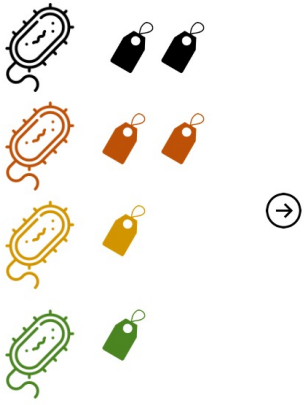
Metabarcoding



Metabarcoding



Metabarcoding

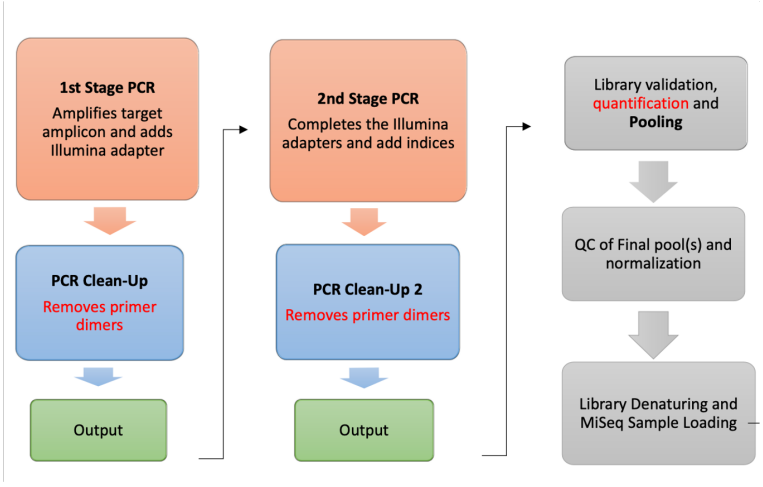


	10	8	10
	4	2	14
	2	10	9
	1	5	88

General workflow

1. Sample collection
2. DNA extraction
3. PCR amplification of our target
4. Adaptor ligation
5. Sequencing
6. Data analysis

Library preparation workflow

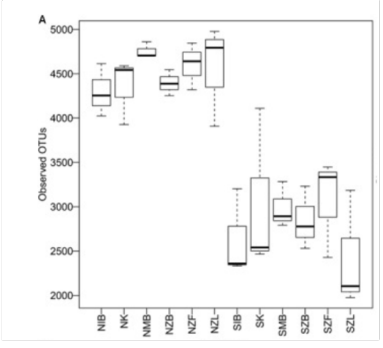


Technical considerations - experimental design

- Target gene (specificity, resolution)
- Replicates (technical/biological)
- Sequencing depth
- Databases for taxonomy assignment
- Bias

Technical considerations - DNA extraction

- DNA extraction introduces a bias in the final dataset
- A recent investigation on 322 studies shows they used 72 different methods. 14 did not report such info!



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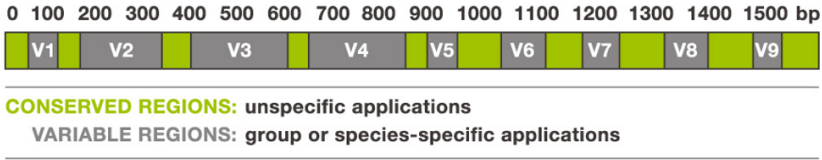
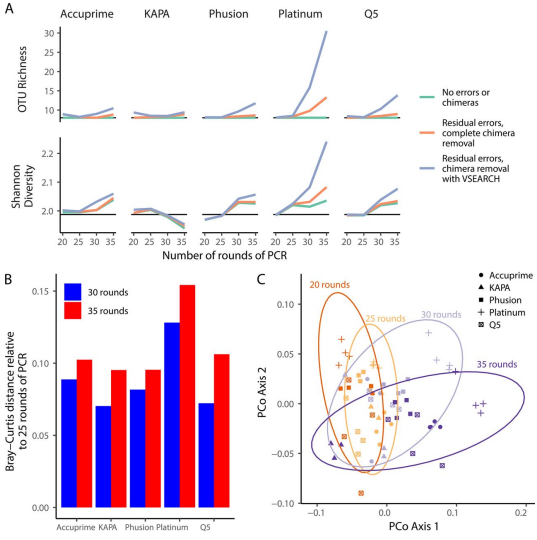
Technical considerations - PCR

- Target gene
  - Who is our target? Bacteria, fungi, insects, fish, specific genus...
  - Is the resolution optimal for our question?
  - Are PCR primers available or we have to design them?
  - Is the taxonomy database available or we have to build a custom one?
- PCR bias
  - Use a Hi-Fi polymerase
  - Use optimal annealing temperature
  - Do not exaggerate with PCR cycles
  - Run multiple PCRs on the same sample



Technical considerations - PCR

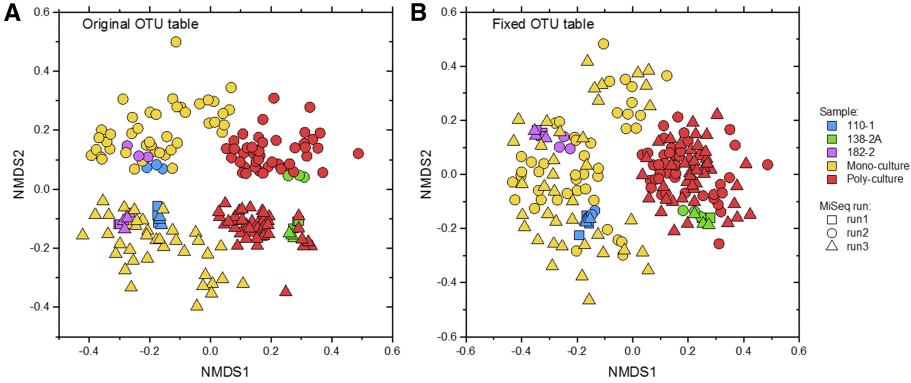


[1] Sze and Schloss 2019 mSphere

Technical considerations - Library prep

- Which sequencing methodology will be a good fit for your research question? (read length/depth of sampling)
- How diverse if your system?

Technical considerations - Sequencing run



[1]Song et al. 2018 Phytobiomes

Control samples

- **Negative control 1.** Run molecular biology grade water throughout the pipeline. Pool it with the other samples even if you do not see amplification.
- **Negative control 2.** This is the negative control from your first PCR. If you see a band, discard the entire batch of samples and start again. If no band is observed, sequence anyway.
- **Mock community.** Pre-built or custom.

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