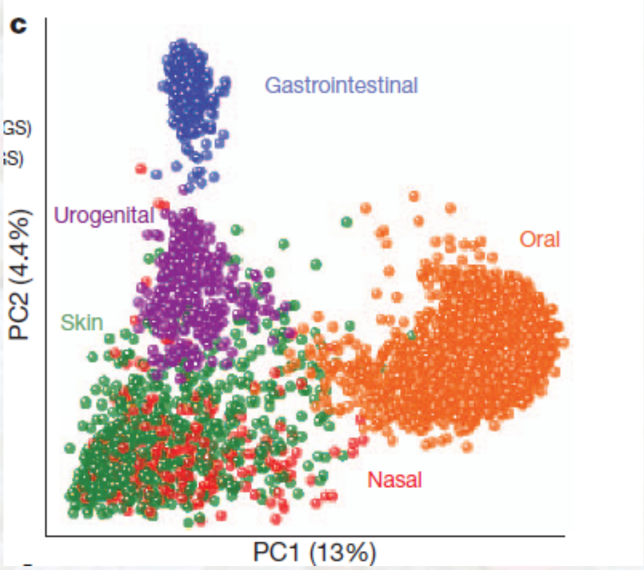
Lecture 01:
Overview of Metagenomics



Culture Independent Techniques:

Metagenomics

Number of Species Counted

- Universal Gene census
- Shotgun Metagenome Sequencing
- Transcriptomics (shotgun mRNA)
- Proteomics (protein fragments)
- Metabolomics (excreted chemicals)

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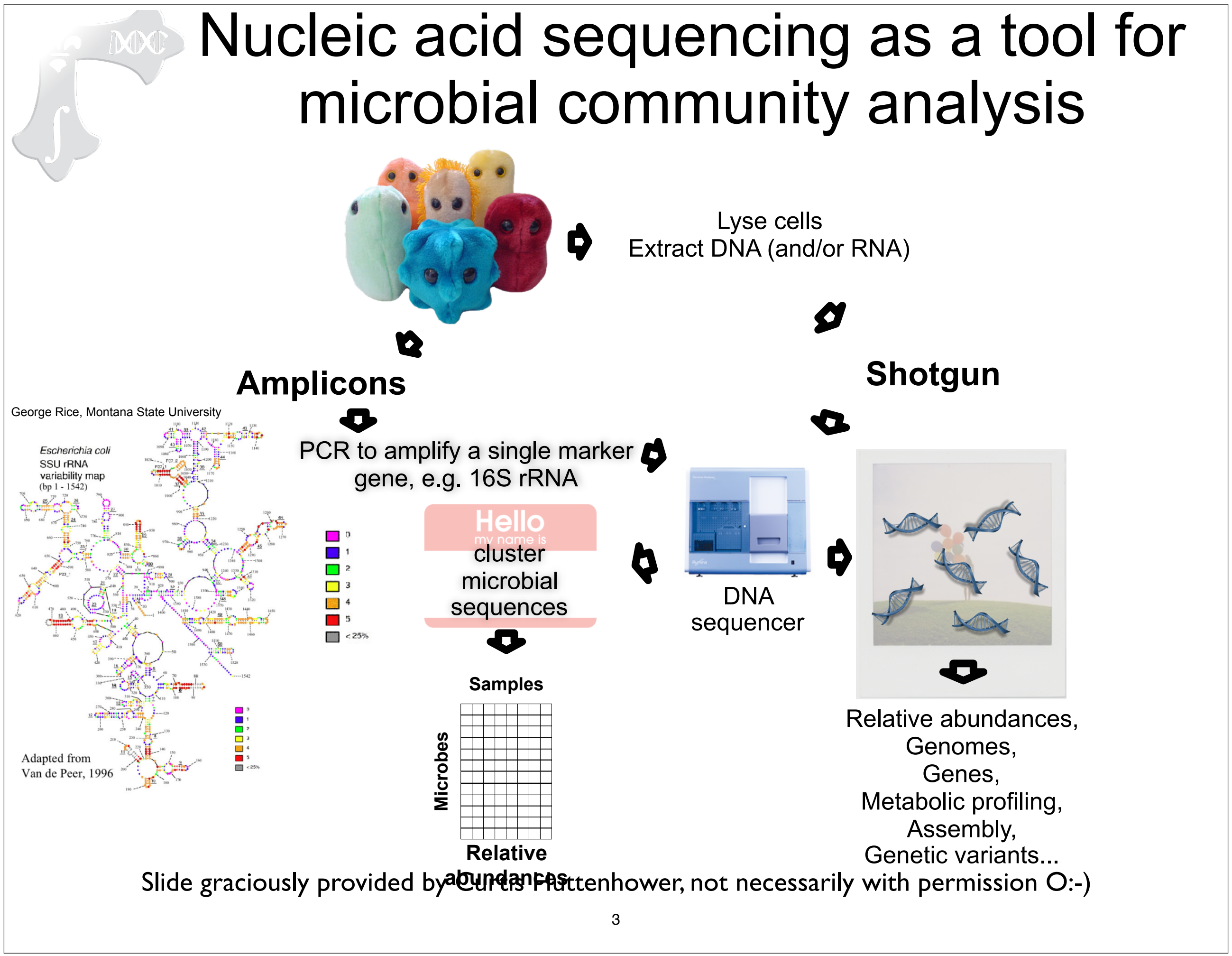

Nucleic acid sequencing as a tool for microbial community analysis

Lyse cells
Extract DNA (and/or RNA)

Amplicons

Shotgun

George Rice, Montana State University

Adapted from
Van de Peer, 1996

PCR to amplify a single marker
gene, e.g. 16S rRNA

cluster
microbial
sequences

DNA
sequencer

Samples

Microbes

Relative abundances

Relative abundances,
Genomes,
Genes,
Metabolic profiling,
Assembly,
Genetic variants...

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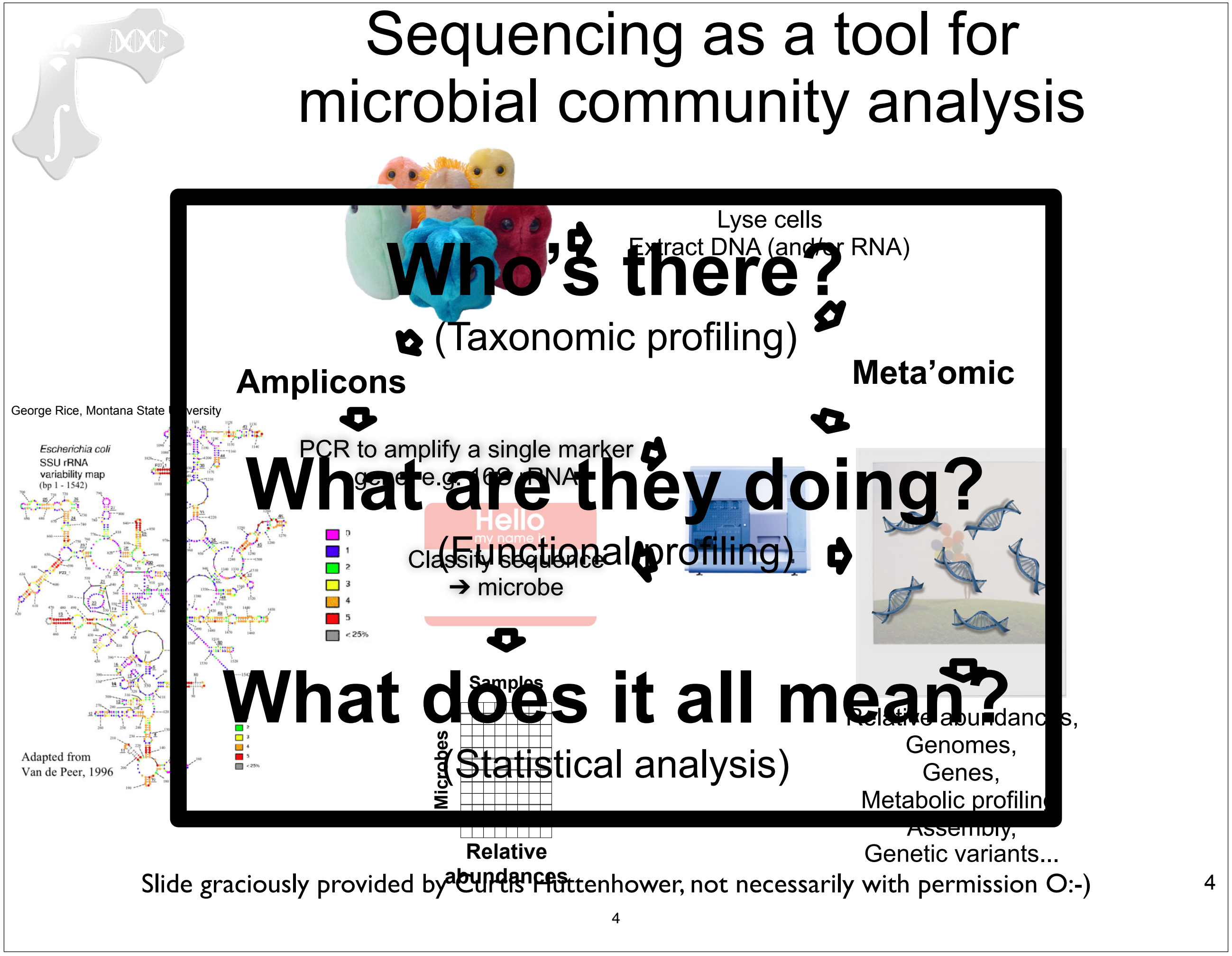

Sequencing as a tool for microbial community analysis

Lyse cells
Extract DNA (and/or RNA)

Who's there?

(Taxonomic profiling)

Amplicons

Meta'omic

George Rice, Montana State University

Adapted from
Van de Peer, 1996

PCR to amplify a single marker
gene, e.g. 16S rRNA

What are they doing?

(Functional profiling)

Classify sequence
➔ microbe

What does it all mean?

(Statistical analysis)

Samples

Microbes

Relative abundances

Relative abundances,
Genomes,
Genes,
Metabolic profiling,
Assembly,
Genetic variants...

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4

A Summary of Meta'omics

Piles of short DNA/RNA reads from >1 organism

You can...

- Ecologically profile them
- Taxonomically or phylogenetically profile them
- Functionally profile them – gene/pathway catalogs
- Assemble them

Prior knowledge is helpful

Caution: Correlation ≠ Causation

- Most 'omics results require lab confirmation

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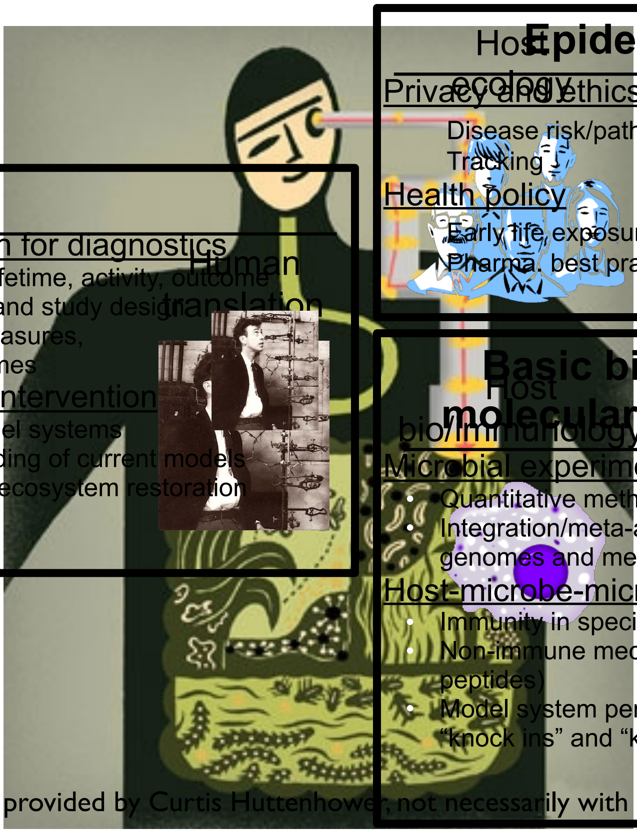
Working toward high-impact outcomes from meta'omic microbial community profiling



Epidemiology

Host ecology

Microbial ecology

Privacy and ethics

- Disease risk/pathogen exposure
- Tracking

Health policy

- Early life exposures
- Pharma. best practices

Translation

Human translation

Phenotype association for diagnostics

- Human disease risk: lifetime, activity, outcome
- Longitudinal analysis and study design
- Dense longitudinal measures, multiple nested outcomes

Systems analysis for intervention

- More and simpler model systems
- Systematic understanding of current models
- Ecological models for ecosystem restoration

Basic biology and molecular mechanism

Host bio/immunology

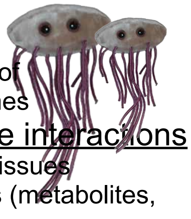
Microbiology

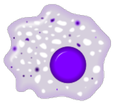
Microbial experiments

- Quantitative methods
- Integration/meta-analysis of genomes and metagenomes

Host-microbe-microbiome interactions

- Immunity in specific host tissues
- Non-immune mechanisms (metabolites, peptides)
- Model system perturbations, "knock ins" and "knock outs"





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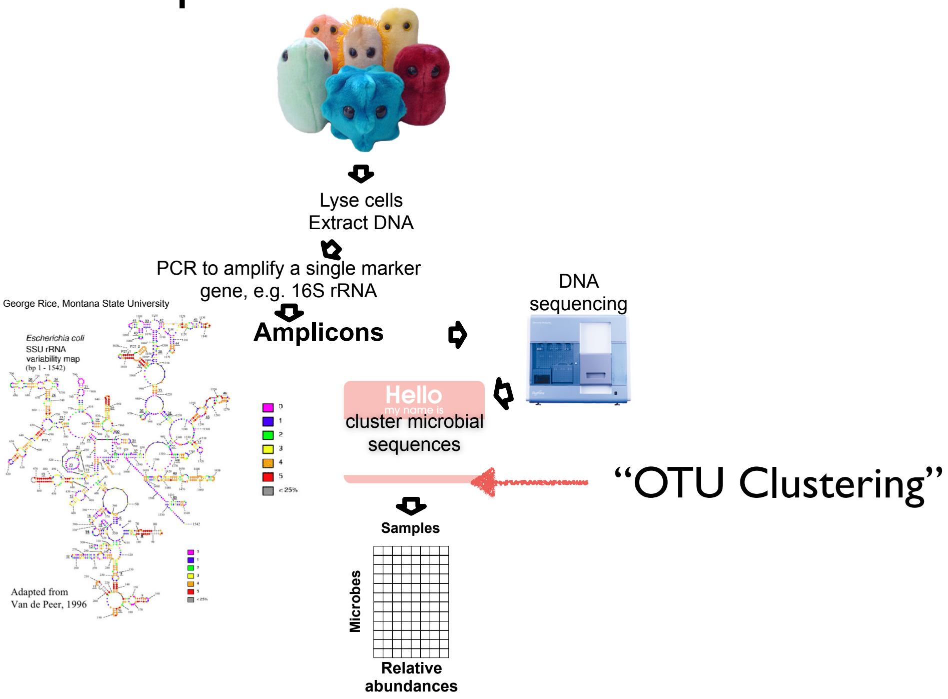
- **Sequence Processing (OTUs)**
  - Denoising
  - Chimera detection
  - Construction of sequence clusters (OTUs)
- **Comparing microbiomes**
  - Distances, Diversity
  - Exploratory Data Analysis
    - Ordination Methods
    - hierarchical dendrogram
    - extract patterns from a plot
      - clusters - gap statistic
      - gradient - regression, modeling, etc.
- **Identifying important microbes/taxa**
  - projected points, coinertia (plots)
  - inferential testing
  - modeling

OTUs - Operational Taxonomic Unit


Lyse cells
Extract DNA
PCR to amplify a single marker gene, e.g. 16S rRNA
George Rice, Montana State University
Escherichia coli SSU rRNA variability map (bp 1 - 1542)
Adapted from Van de Peer, 1996
Amplicons
DNA sequencing
Hello
my name is
cluster microbial sequences
"OTU Clustering"
Samples
Microbes
Relative abundances

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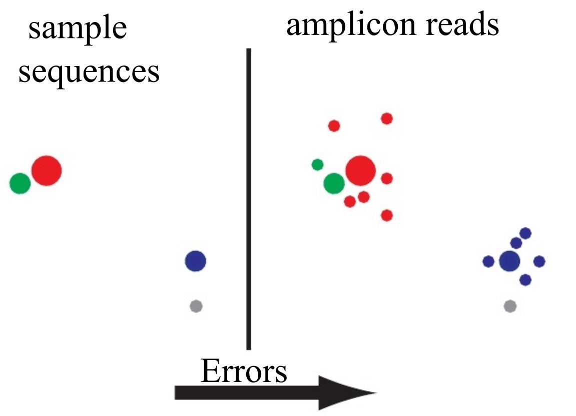
Motivation: Lingering problem with “OTUs”

Some lingering major problems with OTU approaches:

- **False Positives - e.g. 1000s of OTUs when only 10s of strains present**
- **Low Resolution - defined by arbitrary similarity radius**
- **Scaling to large datasets, comparisons**
  - **scales ~ N^2 unique sequences in dataset (all libraries)**
- **Unstable - OTU seq and count depends on input**
  - **must re-run clustering if any data added/removed, or**
  - **if you want to compare against an external dataset**



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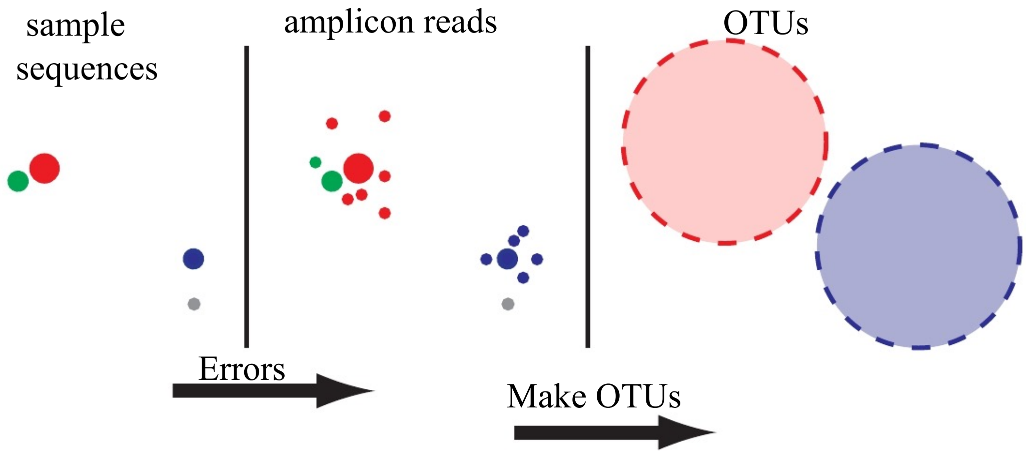

sample sequences

amplicon reads

OTUs

Errors

Make OTUs

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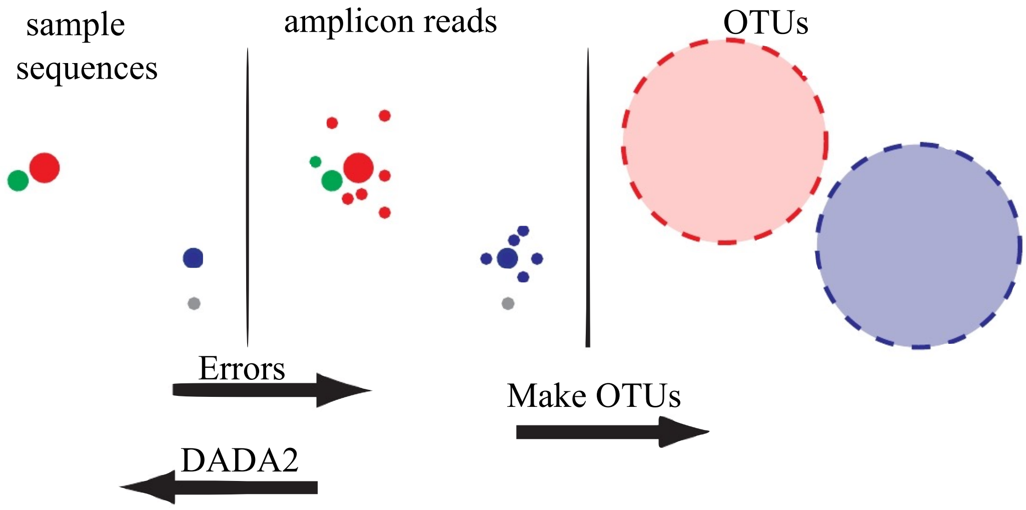
Sample Inference from Noisy Reads



sample sequences

amplicon reads

OTUs

Errors

Make OTUs

DADA2

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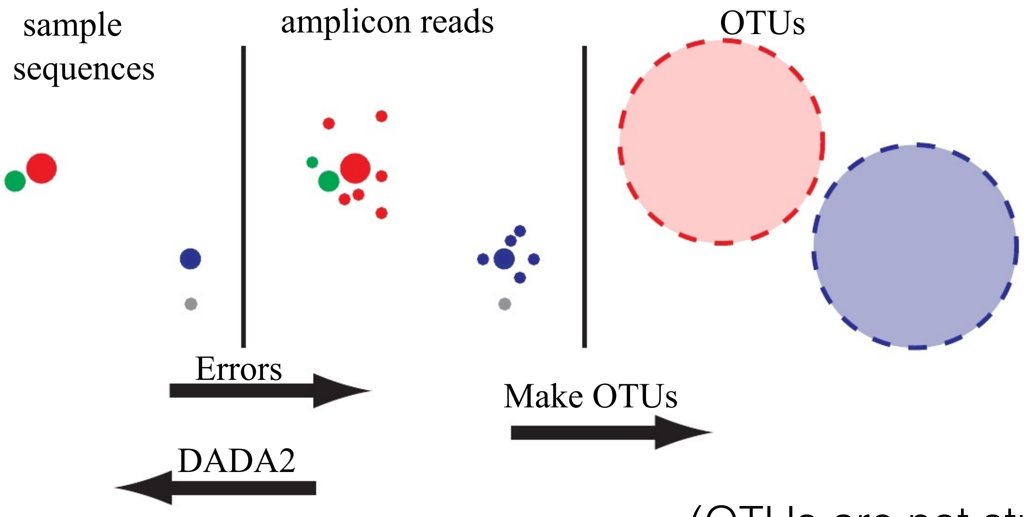
Sample Inference from Noisy Reads



(OTUs are not strains)

OTUs: Lump similar sequences together
DADA2: Statistically infer the sample sequences (strains)

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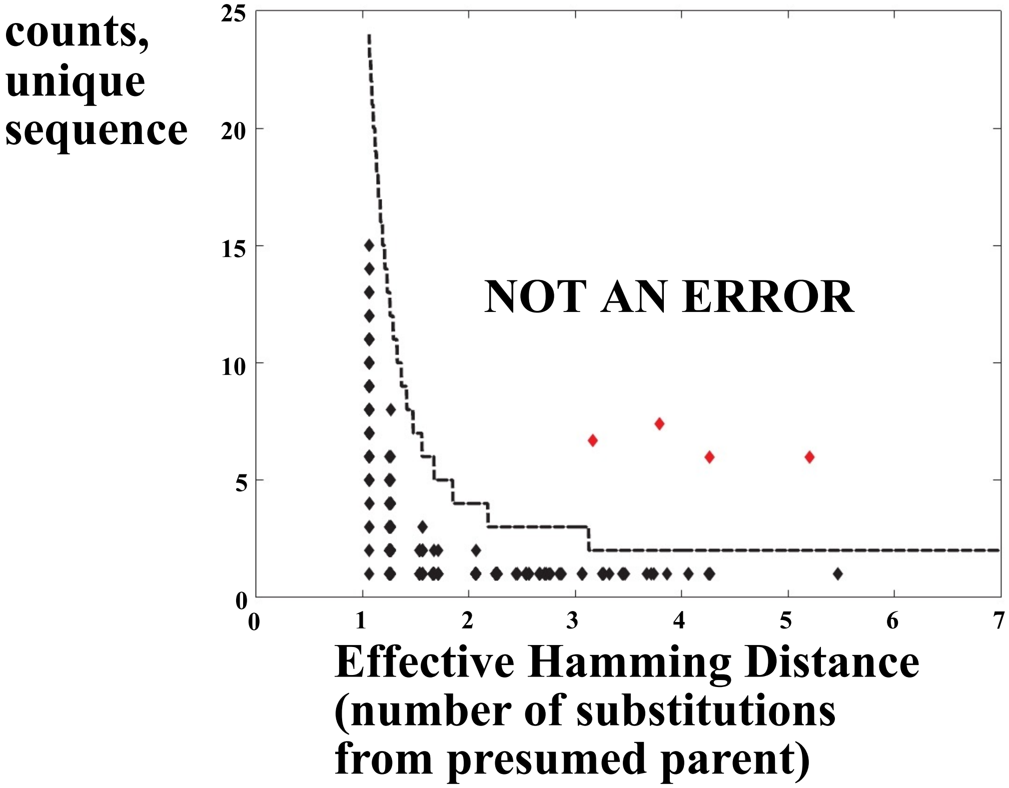
The true shape of an error cloud

DADA2: Error Model



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DADA2 algorithm assumptions

DADA2 Error Model

DADA2 algorithm assumptions

DADA2 Error Model

- Errors independent b/w different sequences
- Errors independent b/w sites within a sequence
- Errant sequence i is produced from j with probability equal to the product of site-wise transition probabilities:

$$\lambda_{j \to i} = \prod_{l=0}^{L} p(j(l) \to i(l), q(l))$$

- Each transition probability depends on original nt, substituting nt, and quality score

DADA2 algorithm assumptions

DADA2 Abundance Model

DADA2 algorithm assumptions

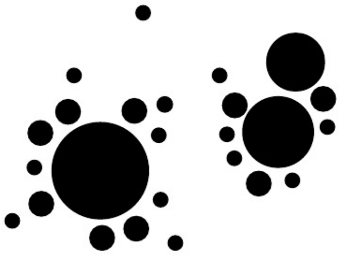
DADA2 Abundance Model

- Errors are independent across reads
- Abundance of reads w/ sequence i produced from more-abundant sequence j is poisson distributed
- Expectation of abundance equals error rate, $\lambda j \to i$, multiplied by the expected reads of sample sequence j
- i has count greater than or equal to one
- “Abundance p-value” for sequence i is thus:

$$p_A(j \to i) = \sum_{a=a_i}^{\infty} \rho_{pois}(n_j \lambda_{j\to i}, a)/(1 - \rho_{pois}(n_j \lambda_{j\to i}, 0))$$

- “Probability of seeing an abundance of sequence i that is equal to or greater than observed value, by chance, given sequence j.”
- A low p_A indicates that there are more reads of sequence i than can be explained by errors introduced during the amplification and sequencing of n_j copies

DADA2 algorithm cartoon



Initial guess: one real sequence + errors

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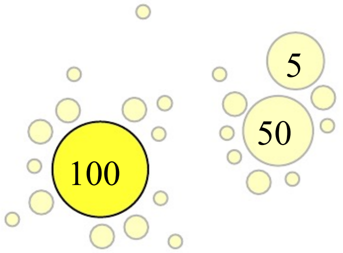
DADA2 algorithm cartoon



Infer initial *error model* under this assumption.

$\Pr(i \rightarrow j) =$

	A	C	G	T
A	0.97	10_{-2}	10_{-2}	10_{-2}
C	10_{-2}	0.97	10_{-2}	10_{-2}
G	10_{-2}	10_{-2}	0.97	10_{-2}
T	10_{-2}	10_{-2}	10_{-2}	0.97

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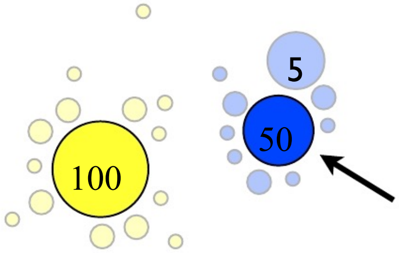
DADA2 algorithm cartoon



not an error

Reject unlikely error under model. **Recruit** errors.

	A	C	G	T
A	0.97	10_{-2}	10_{-2}	10_{-2}
C	10_{-2}	0.97	10_{-2}	10_{-2}
G	10_{-2}	10_{-2}	0.97	10_{-2}
T	10_{-2}	10_{-2}	10_{-2}	0.97

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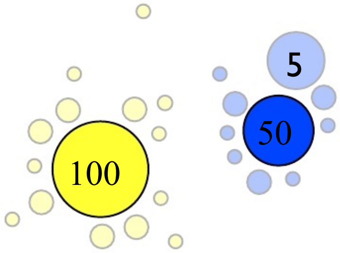
DADA2 algorithm cartoon



Update the model.

	A	C	G	T
A	0.997	10_{-3}	10_{-3}	10_{-3}
C	10_{-3}	0.997	10_{-3}	10_{-3}
G	10_{-3}	10_{-3}	0.997	10_{-3}
T	10_{-3}	10_{-3}	10_{-3}	0.997

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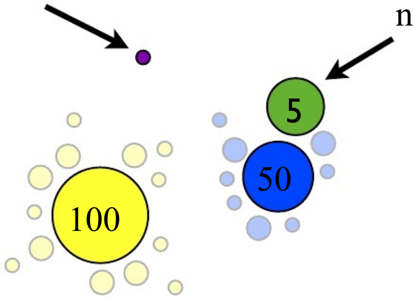
DADA2 algorithm cartoon

not an error



not an error

Reject more sequences under ***new*** model

	A	C	G	T
A	0.997	10_{-3}	10_{-3}	10_{-3}
C	10_{-3}	0.997	10_{-3}	10_{-3}
G	10_{-3}	10_{-3}	0.997	10_{-3}
T	10_{-3}	10_{-3}	10_{-3}	0.997

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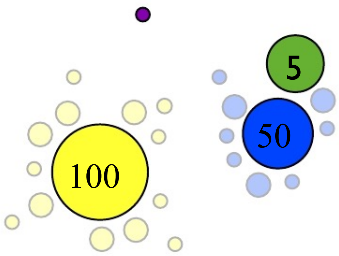
DADA2 algorithm cartoon



Update model again

	A	C	G	T
A	0.998	$1x10_{-4}$	$2x10_{-3}$	$2x10_{-4}$
C	$6x10_{-5}$	0.999	$3x10_{-6}$	$1x10_{-3}$
G	$1x10_{-3}$	$3x10_{-6}$	0.999	$6x10_{-5}$
T	$2x10_{-4}$	$2x10_{-3}$	$1x10_{-4}$	0.998

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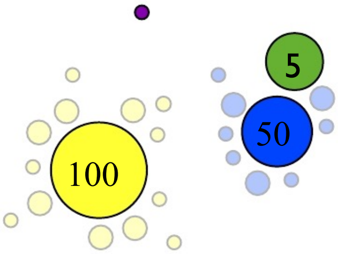
DADA2 algorithm cartoon



Convergence: all errors are plausible

	A	C	G	T
A	0.998	$1x10^{-4}$	$2x10^{-3}$	$2x10^{-4}$
C	$6x10^{-5}$	0.999	$3x10^{-6}$	$1x10^{-3}$
G	$1x10^{-3}$	$3x10^{-6}$	0.999	$6x10^{-5}$
T	$2x10^{-4}$	$2x10^{-3}$	$1x10^{-4}$	0.998

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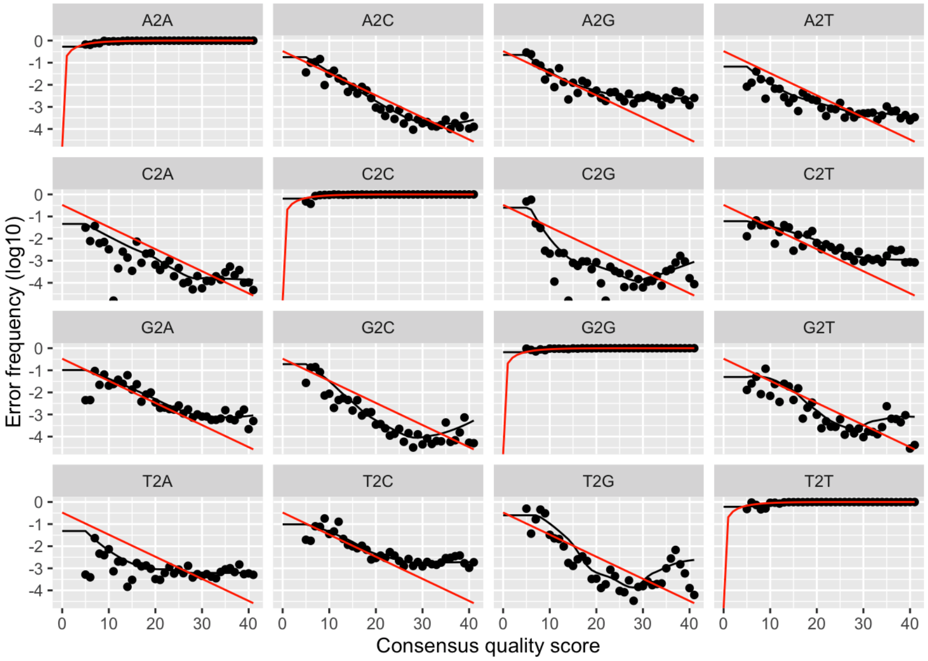

- *selfConsist* mode for DADA2 includes joint inference of error rates as function of quality score.
- red line is expected error rate if Q-scores were exactly correct
- black line is DADA2's empirical model (smooth)
- Notice especially overestimate of errors at high values, Q >30
- For illumina these differences are specific to sequencing run and read direction
  - for small lib sizes, can aggregate estimate across libraries from the same run/direction

DADA2: Why is this possible?

Uses more of the information than traditional OTU clustering

	DADA2	OTUs
Abundance	✓	**Ranks only**
Sequence Differences	✓	**Count only**
Quality	✓	**No**
Error Model	✓	**No**

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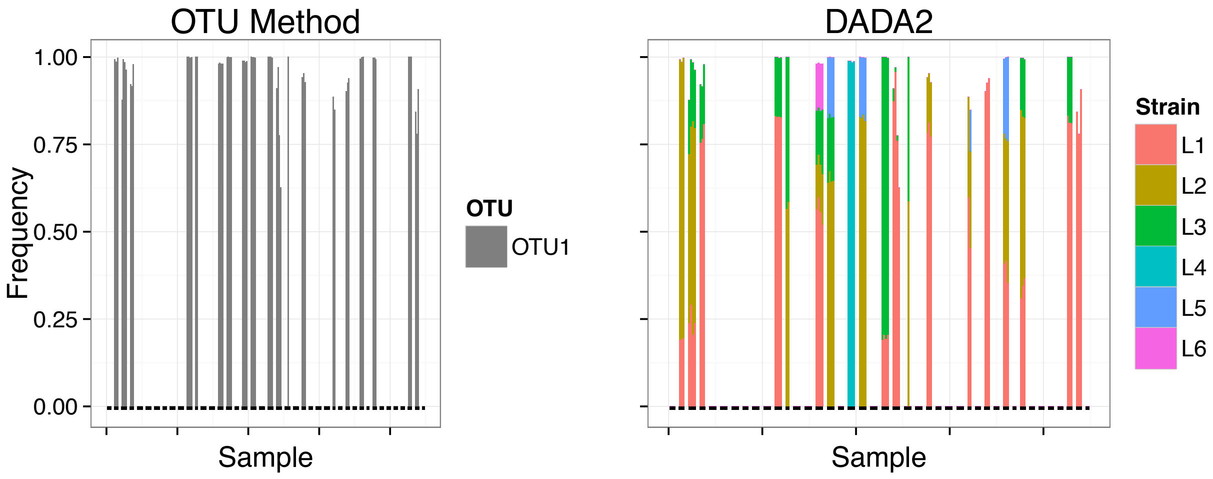
DADA2 Advantages: Resolution

Lactobacillus crispatus sampled from
vaginal microbiome 42 pregnant women



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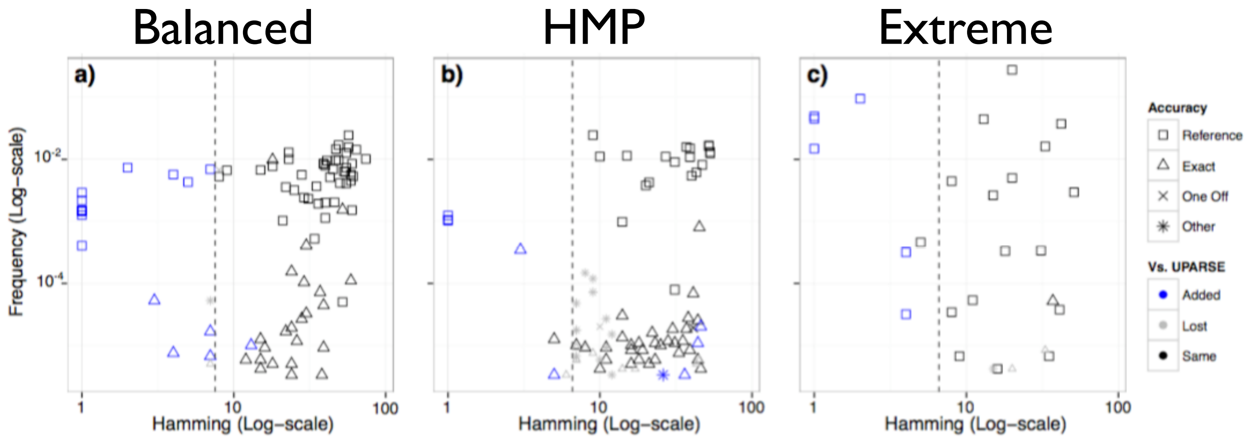
DADA2 Advantages: Accuracy benchmarks

Mock community data for accuracy benchmarking



Balanced

HMP

Extreme

DADA2 performance relative to UPARSE
(best available alternative)

DADA2 Advantages

Analytical

Single nucleotide resolution
- genotypes/strains instead of 97% OTUs

Lower false positive rate
- Better error model, easier to ID chimeras

Computational

Linear scaling of computational costs
- Exact sequences are inherently comparable, so samples can be processed independently.

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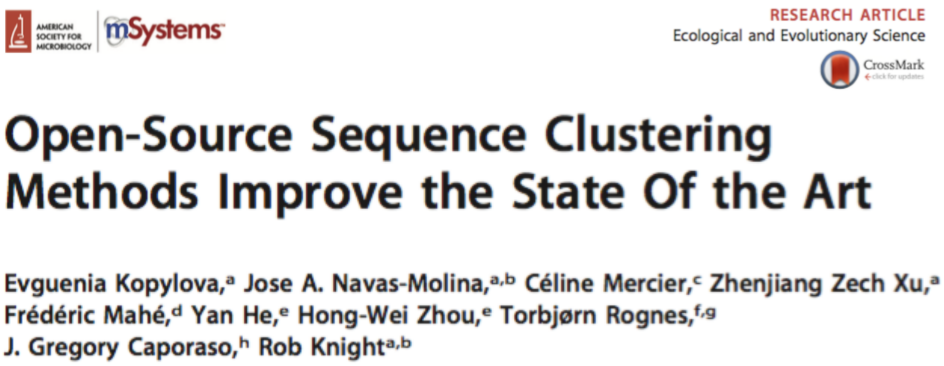

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RESEARCH ARTICLE
Ecological and Evolutionary Science

CrossMark

Open-Source Sequence Clustering Methods Improve the State Of the Art

Evguenia Kopylova,[a] Jose A. Navas-Molina,[a,b] Céline Mercier,[c] Zhenjiang Zech Xu,[a] Frédéric Mahé,[d] Yan He,[e] Hong-Wei Zhou,[e] Torbjørn Rognes,[f,g] J. Gregory Caporaso,[h] Rob Knight[a,b]

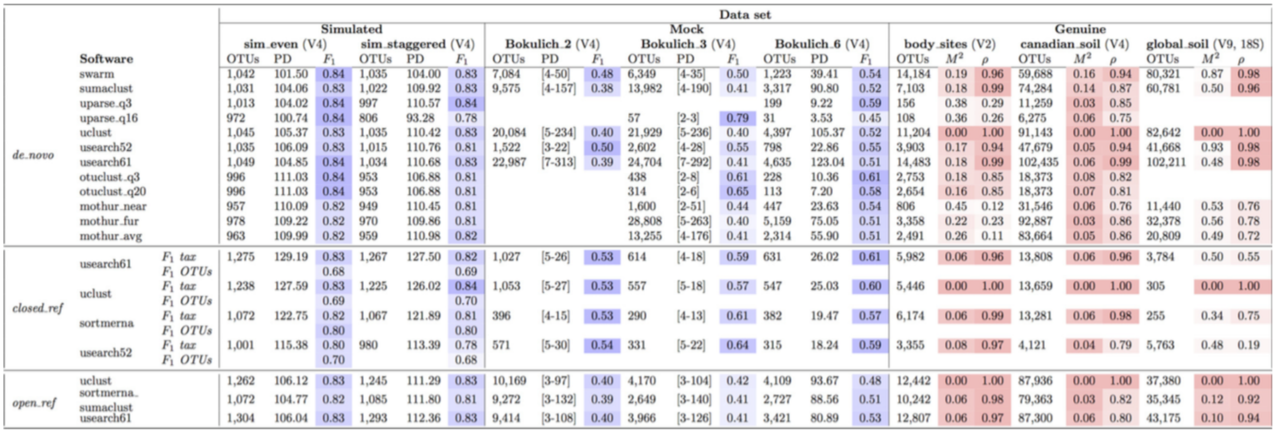
February2016

Four new open-source amplicon-clustering methods in last two years (since UPARSE):

- Swarm - very fast single-linkage clustering unsupervised
- SUMACLUST - abundance-rank greedy clustering unsupervised
- OTUCLUST - abundance-rank greedy clustering unsupervised
- SortMeRNA - clustering after reference alignment supervised

compared mainly against UPARSE (not open-source)

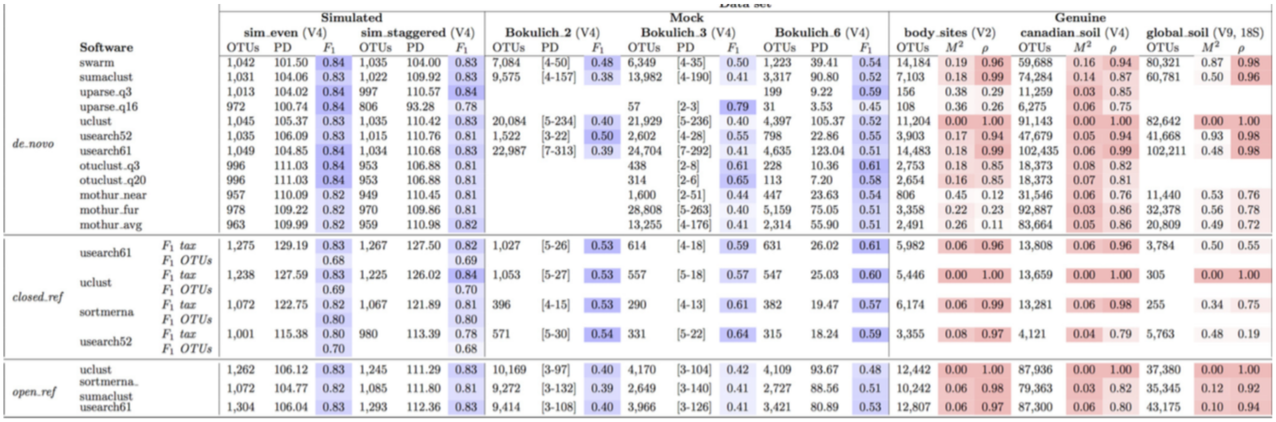
Kopylova, et al (2016).
Open-source sequence clustering methods improve the state of the art.
mSystems
http://doi.org/10.1186/s12915-014-0069-1



			Data set																							
			Simulated						Mock									Genuine								
			sim_even (V4)			sim_staggered (V4)			Bokulich_2 (V4)			Bokulich_3 (V4)			Bokulich_6 (V4)			body_sites (V2)			canadian_soil (V4)			global_soil (V9, 18S)		
	Software		OTUs	PD	F_1	OTUs	PD	F_1	OTUs	PD	F_1	OTUs	PD	F_1	OTUs	PD	F_1	OTUs	M^2	ρ	OTUs	M^2	ρ	OTUs	M^2	ρ
de_novo	swarm		1,042	101.50	0.84	1,035	104.00	0.83	7,084	[4-50]	0.48	6,349	[4-35]	0.50	1,223	39.41	0.54	14,184	0.19	0.96	59,688	0.16	0.94	80,321	0.87	0.98
	sumaclust		1,031	104.06	0.83	1,022	109.92	0.83	9,575	[4-157]	0.38	13,982	[4-190]	0.41	3,317	90.80	0.52	7,103	0.18	0.99	74,284	0.14	0.87	60,781	0.50	0.96
	uparse_q3		1,013	104.02	0.84	997	110.57	0.84							199	9.22	0.59	156	0.38	0.29	11,259	0.03	0.85			
	uparse_q16		972	100.74	0.84	806	93.28	0.78				57	[2-3]	0.79	31	3.53	0.45	108	0.36	0.26	6,275	0.06	0.75			
	uclust		1,045	105.37	0.83	1,035	110.42	0.83	20,084	[5-234]	0.40	21,929	[5-236]	0.40	4,397	105.37	0.52	11,204	0.00	1.00	91,143	0.00	1.00	82,642	0.00	1.00
	usearch52		1,035	106.09	0.83	1,015	110.76	0.81	1,522	[3-22]	0.50	2,602	[4-28]	0.55	798	22.86	0.55	3,903	0.17	0.94	47,679	0.05	0.94	41,668	0.93	0.98
	usearch61		1,049	104.85	0.84	1,034	110.68	0.83	22,987	[7-313]	0.39	24,704	[7-292]	0.41	4,635	123.04	0.51	14,483	0.18	0.99	102,435	0.06	0.99	102,211	0.48	0.98
	otuclust_q3		996	111.03	0.84	953	106.88	0.81				438	[2-8]	0.61	228	10.36	0.61	2,753	0.18	0.85	18,373	0.08	0.82			
	otuclust_q20		996	111.03	0.84	953	106.88	0.81				314	[2-6]	0.65	113	7.20	0.58	2,654	0.16	0.85	18,373	0.07	0.81			
	mothur_near		957	110.09	0.82	949	110.45	0.81				1,600	[2-51]	0.44	447	23.63	0.54	806	0.45	0.12	31,546	0.06	0.76	11,440	0.53	0.76
	mothur_fur		978	109.22	0.82	970	109.86	0.81				28,808	[5-263]	0.40	5,159	75.05	0.51	3,358	0.22	0.23	92,887	0.03	0.86	32,378	0.56	0.78
	mothur_avg		963	109.99	0.82	959	110.98	0.82				13,255	[4-176]	0.41	2,314	55.90	0.51	2,491	0.26	0.11	83,664	0.05	0.86	20,809	0.49	0.72
closed_ref	usearch61	F_1 *tax*	1,275	129.19	0.83	1,267	127.50	0.82	1,027	[5-26]	0.53	614	[4-18]	0.59	631	26.02	0.61	5,982	0.06	0.96	13,808	0.06	0.96	3,784	0.50	0.55
		F_1 *OTUs*			0.68			0.69																		
	uclust	F_1 *tax*	1,238	127.59	0.83	1,225	126.02	0.84	1,053	[5-27]	0.53	557	[5-18]	0.57	547	25.03	0.60	5,446	0.00	1.00	13,659	0.00	1.00	305	0.00	1.00
		F_1 *OTUs*			0.69			0.70																		
	sortmerna	F_1 *tax*	1,072	122.75	0.82	1,067	121.89	0.81	396	[4-15]	0.53	290	[4-13]	0.61	382	19.47	0.57	6,174	0.06	0.99	13,281	0.06	0.98	255	0.34	0.75
		F_1 *OTUs*			0.80			0.80																		
	usearch52	F_1 *tax*	1,001	115.38	0.80	980	113.39	0.78	571	[5-30]	0.54	331	[5-22]	0.64	315	18.24	0.59	3,355	0.08	0.97	4,121	0.04	0.79	5,763	0.48	0.19
		F_1 *OTUs*			0.70			0.68																		
open_ref	uclust		1,262	106.12	0.83	1,245	111.29	0.83	10,169	[3-97]	0.40	4,170	[3-104]	0.42	4,109	93.67	0.48	12,442	0.00	1.00	87,936	0.00	1.00	37,380	0.00	1.00
	sortmerna_ sumaclust		1,072	104.77	0.82	1,085	111.80	0.81	9,272	[3-132]	0.39	2,649	[3-140]	0.41	2,727	88.56	0.51	10,242	0.06	0.98	79,363	0.03	0.82	35,345	0.12	0.92
	usearch61		1,304	106.04	0.83	1,293	112.36	0.83	9,414	[3-108]	0.40	3,966	[3-126]	0.41	3,421	80.89	0.53	12,807	0.06	0.97	87,300	0.06	0.80	43,175	0.10	0.94

(OTU counts do not include singletons)

Kopylova, et al (2016).
Open-source sequence clustering methods improve the state of the art.
mSystems
http://doi.org/10.1186/s12915-014-0069-1



			Data set																							
			Simulated						Mock									Genuine								
			sim_even (V4)			sim_staggered (V4)			Bokulich_2 (V4)			Bokulich_3 (V4)			Bokulich_6 (V4)			body_sites (V2)			canadian_soil (V4)			global_soil (V9, 18S)		
	Software		OTUs	PD	F_1	OTUs	PD	F_1	OTUs	PD	F_1	OTUs	PD	F_1	OTUs	PD	F_1	OTUs	M^2	ρ	OTUs	M^2	ρ	OTUs	M^2	ρ
de_novo	swarm		1,042	101.50	0.84	1,035	104.00	0.83	7,084	[4-50]	0.48	6,349	[4-35]	0.50	1,223	39.41	0.54	14,184	0.19	0.96	59,688	0.16	0.94	80,321	0.87	0.98
	sumaclust		1,031	104.06	0.83	1,022	109.92	0.83	9,575	[4-157]	0.38	13,982	[4-190]	0.41	3,317	90.80	0.52	7,103	0.18	0.99	74,284	0.14	0.87	60,781	0.50	0.96
	uparse_q3		1,013	104.02	0.84	997	110.57	0.84							199	9.22	0.59	156	0.38	0.29	11,259	0.03	0.85			
	uparse_q16		972	100.74	0.84	806	93.28	0.78				57	[2-3]	0.79	31	3.53	0.45	108	0.36	0.26	6,275	0.06	0.75			
	uclust		1,045	105.37	0.83	1,035	110.42	0.83	20,084	[5-234]	0.40	21,929	[5-236]	0.40	4,397	105.37	0.52	11,204	0.00	1.00	91,143	0.00	1.00	82,642	0.00	1.00
	usearch52		1,035	106.09	0.83	1,015	110.76	0.81	1,522	[3-22]	0.50	2,602	[4-28]	0.55	798	22.86	0.55	3,903	0.17	0.94	47,679	0.05	0.94	41,668	0.93	0.98
	usearch61		1,049	104.85	0.84	1,034	110.68	0.83	22,987	[7-313]	0.39	24,704	[7-292]	0.41	4,635	123.04	0.51	14,483	0.18	0.99	102,435	0.06	0.99	102,211	0.48	0.98
	otuclust_q3		996	111.03	0.84	953	106.88	0.81				438	[2-8]	0.61	228	10.36	0.61	2,753	0.18	0.85	18,373	0.08	0.82			
	otuclust_q20		996	111.03	0.84	953	106.88	0.81				314	[2-6]	0.65	113	7.20	0.58	2,654	0.16	0.85	18,373	0.07	0.81			
	mothur_near		957	110.09	0.82	949	110.45	0.81				1,600	[2-51]	0.44	447	23.63	0.54	806	0.45	0.12	31,546	0.06	0.76	11,440	0.53	0.76
	mothur_fur		978	109.22	0.82	970	109.86	0.81				28,808	[5-263]	0.40	5,159	75.05	0.51	3,358	0.22	0.23	92,887	0.03	0.86	32,378	0.56	0.78
	mothur_avg		963	109.99	0.82	959	110.98	0.82				13,255	[4-176]	0.41	2,314	55.90	0.51	2,491	0.26	0.11	83,664	0.05	0.86	20,809	0.49	0.72
closed_ref	usearch61	F_1 *tax*	1,275	129.19	0.83	1,267	127.50	0.82	1,027	[5-26]	0.53	614	[4-18]	0.59	631	26.02	0.61	5,982	0.06	0.96	13,808	0.06	0.96	3,784	0.50	0.55
		F_1 *OTUs*			0.68			0.69																		
	uclust	F_1 *tax*	1,238	127.59	0.83	1,225	126.02	0.84	1,053	[5-27]	0.53	557	[5-18]	0.57	547	25.03	0.60	5,446	0.00	1.00	13,659	0.00	1.00	305	0.00	1.00
		F_1 *OTUs*			0.69			0.70																		
	sortmerna	F_1 *tax*	1,072	122.75	0.82	1,067	121.89	0.81	396	[4-15]	0.53	290	[4-13]	0.61	382	19.47	0.57	6,174	0.06	0.99	13,281	0.06	0.98	255	0.34	0.75
		F_1 *OTUs*			0.80			0.80																		
	usearch52	F_1 *tax*	1,001	115.38	0.80	980	113.39	0.78	571	[5-30]	0.54	331	[5-22]	0.64	315	18.24	0.59	3,355	0.08	0.97	4,121	0.04	0.79	5,763	0.48	0.19
		F_1 *OTUs*			0.70			0.68																		
open_ref	uclust		1,262	106.12	0.83	1,245	111.29	0.83	10,169	[3-97]	0.40	4,170	[3-104]	0.42	4,109	93.67	0.48	12,442	0.00	1.00	87,936	0.00	1.00	37,380	0.00	1.00
	sortmerna_ sumaclust		1,072	104.77	0.82	1,085	111.80	0.81	9,272	[3-132]	0.39	2,649	[3-140]	0.41	2,727	88.56	0.51	10,242	0.06	0.98	79,363	0.03	0.82	35,345	0.12	0.92
	usearch61		1,304	106.04	0.83	1,293	112.36	0.83	9,414	[3-108]	0.40	3,966	[3-126]	0.41	3,421	80.89	0.53	12,807	0.06	0.97	87,300	0.06	0.80	43,175	0.10	0.94

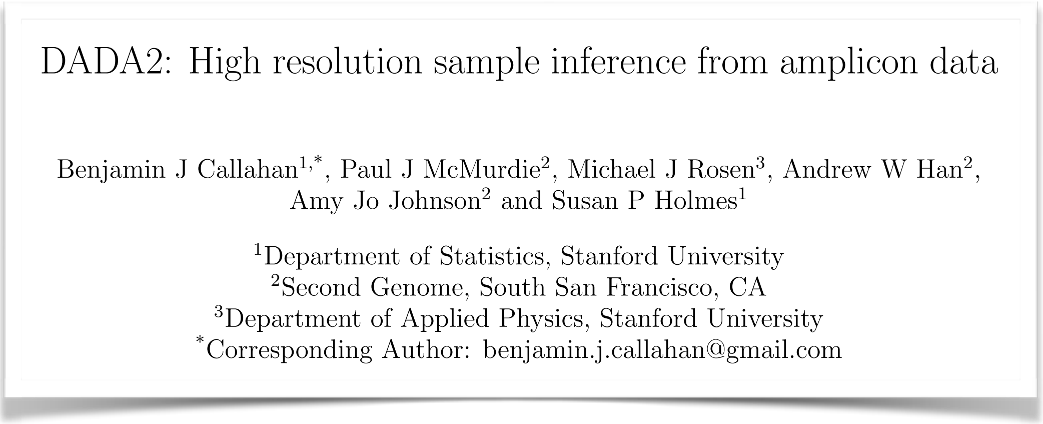
DADA2 performance:

- Mock:
  - Bokulich_6: 64 sequences, 25/26 taxonomies, 6 new
  - Bokulich_2: 17 sequences, 18/18 taxonomies, 11 new
- Simulations:
  - Even: 1055/1055 sequence variants, no Fps
  - Staggered: 1,042/1,055

http://benjjneb.github.io/dada2/R/SotA.html

DADA2

Divisive Amplicon Denoising Algorithm - ver.2



DADA2: High resolution sample inference from amplicon data

Benjamin J Callahan[1,*], Paul J McMurdie[2], Michael J Rosen[3], Andrew W Han[2], Amy Jo Johnson[2] and Susan P Holmes[1]

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http://dx.doi.org/10.1101/024034 Manuscript draft on bioRxiv (*Nature Methods*, in press)

http://benjjneb.github.io/dada2/ R package available on BioConductor

DADA1: Rosen MJ, Callahan BJ, Fisher DS, Holmes SP
(2012) Denoising PCR-amplified metagenome data. BMC bioinformatics, 13(1), 283.

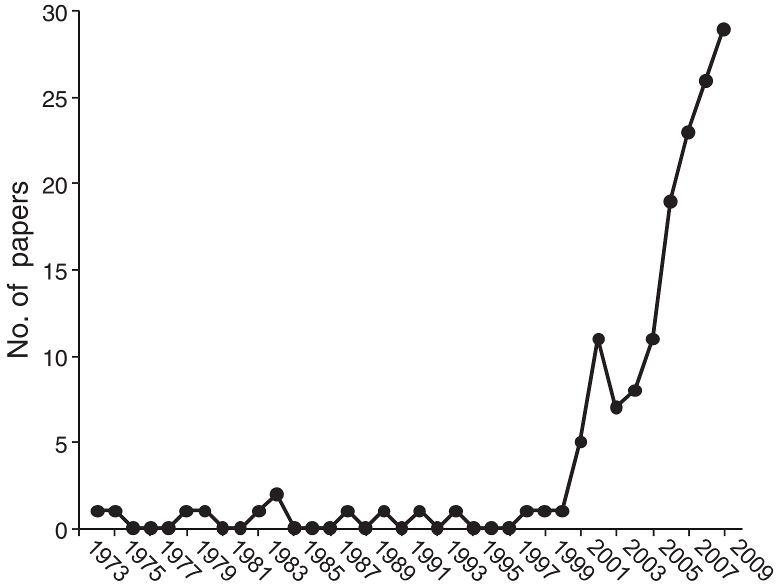
Diversity

Diversity of diversity
(diversity of greek letters used in ecology)

- α – diversity within a community, # of species
- β – diversity between communities (differentiation), species identity is taken into account
- γ – (global) diversity of the site, γ = α × β, but only this simple if α and β are independent
- Probably others, but α and β are most common

Beta-Diversity

Peer-reviewed articles having “beta diversity” in title



Anderson, M. J., et al. (2011). Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. Ecology Letters, 14(1), 19–28.

Beta-Diversity

- Microbial ecologists typically use beta diversity as a broad umbrella term that can refer to any of several indices related to compositional differences

  (Differences in species content between samples)
- For some reason this is contentious, and there appears to be ongoing (and pointless?) argument over the possible definitions
- For our purposes, and microbiome research, when you hear “beta-diversity”, you can probably think:

“Diversity of species composition”

http://en.wikipedia.org/wiki/Beta_diversity

Distances between microbiomes

Community Distance

Communities are a vector of abundances:
$\mathbf{x} = \{x_1, x_2, x_3, \ldots\}$

E. coli: ● ● ●
P. fluorescens: ●
B. subtilis: ●
P. acnes:
D. radiodurans:
H. pylori: ● ● ● ● ● ● ●
L. crispatus:

$\mathbf{x} = \{3,1,1,0,0,7,0\}$

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Community Distance Properties

- Range from 0 to 1
- Distance to self is 0
- If no shared taxa, distance is 1
- Triangle inequality (metric)
- Joint absences do not affect distance (biology)
- Independent of absolute counts (metagenomics)

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The Distance Spectrum

	Categorical	Phylogenetic
Presence/ Absence	Jaccard	Unifrac
Quantitative Abundance	Bray-Curtis	Weighted Unifrac

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The Distance Spectrum

	Categorical	Phylogenetic
Presence/ Absence	Jaccard	Unifrac
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phyloseq distances

manhattan
euclidean
canberra
bray
kulczynski
jaccard
gower
altGower
morisita-horn
mountford
raup
binomial
chao
cao
jensen-shannon
unifrac
weighted-unifrac

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Ordination Methods

Project high-dimensional data onto lower dimensions

P taxa

N samples

0,1,5,1,0,1,2,1,0,0,9,…
7,2,0,0,0,0,0,0,1,0,0,…
0,0,0,0,0,0,8,0,0,0,1,…
0,0,0,1,0,1,2,0,0,0,5,…
0,1,0,2,0,0,0,1,0,0,4,…
0,0,0,1,9,1,2,5,2,0,1,…
0,0,0,0,0,1,2,1,8,0,0,…
0,0,0,0,9,4,0,0,0,0,1,…
.
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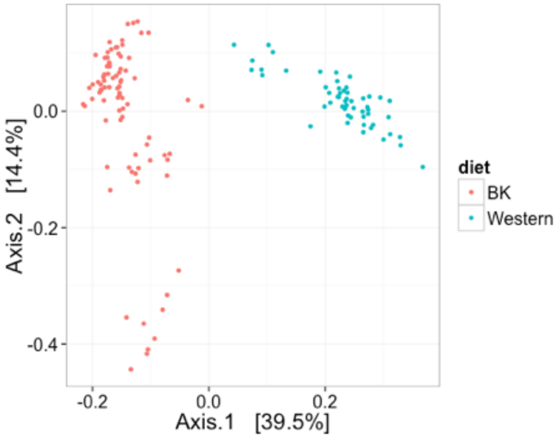

P-dimensions

2-dimensions

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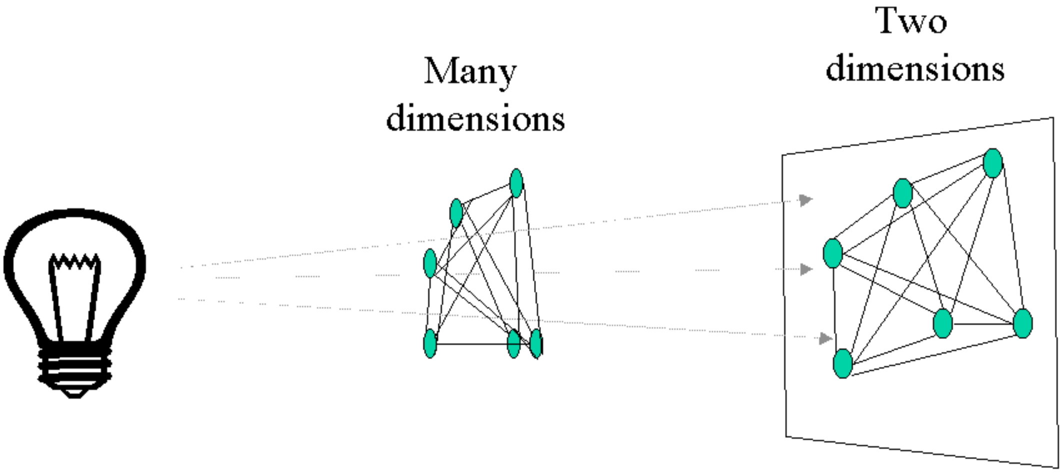
Multi-dimensional Scaling

Why MDS? It works with any distance!



Input distance matrix can by Bray-Curtis, Unifrac, …

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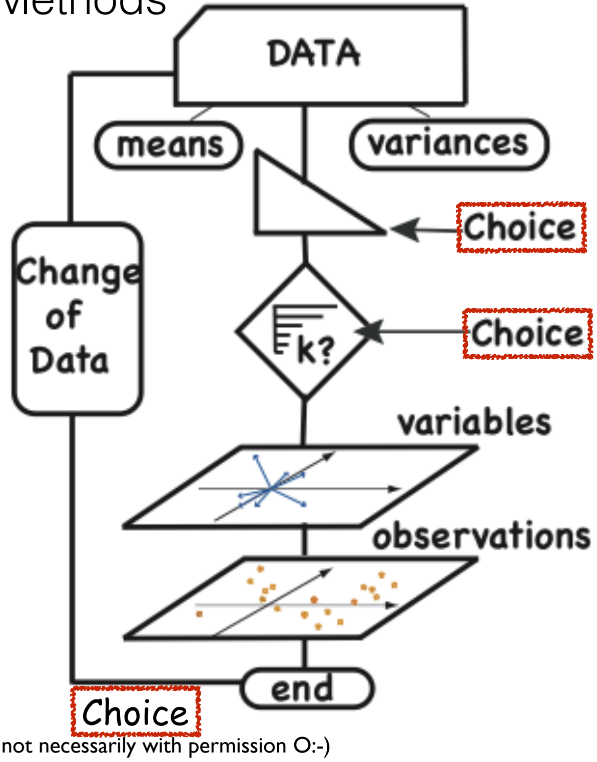
Exploratory Data Analysis

“Unsupervised Learning”
“Ordination Methods”

Best Practices

- Looking for patterns (the “I-test”)
- Always look at scree plot
- Biplot (if legible)
- Use multiple distances
  - For which D is pattern strongest?
- phyloseq (and R/Rmd) make this easy!



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Exploratory Data Analysis

“Unsupervised Learning”
“Ordination Methods”

What we “learn” depends on the data.

- How many axes are probably useful?
- Are there clusters? How many?
- Are there gradients?
- Are the patterns consistent with covariates
- (e.g. sample observations)
- How might we test this?