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RECYCLES WORKSHOP

Metagenomics and
metabarcoding
approaches to describe
ecological systems and
infer their development

5th, 6th & 7th of July 2022

**Primers relevance in metabarcoding:
an example for anammox reactors**

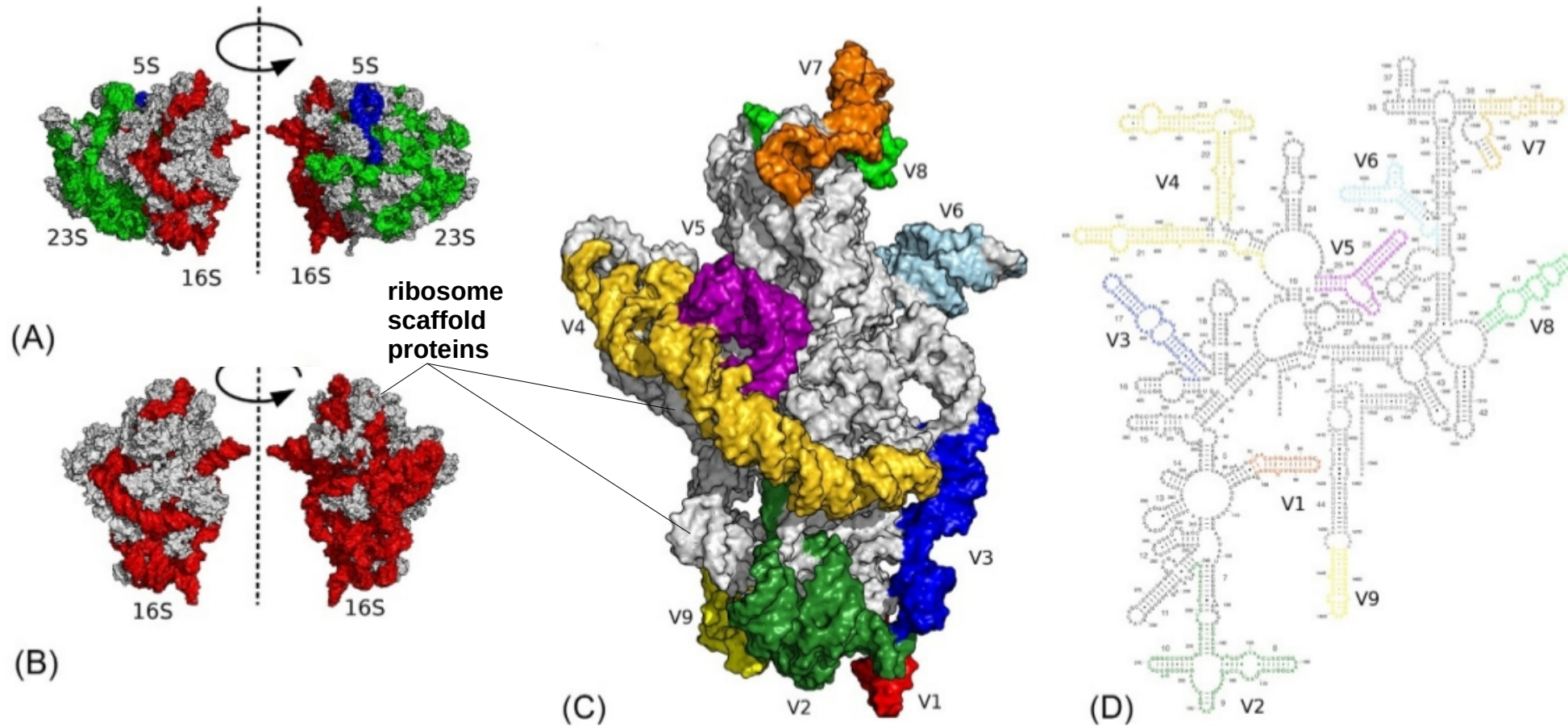
Matteo Ramazzotti





- **Ribosomes and their conservation**
- **Targeting rRNA with PCR**
- **Choice of the V region**
- **V3-V4 primers**
- **Prediction of coverage**
- **Brocadia-aware new primer**

Ribosome as a ribonucleoprotein



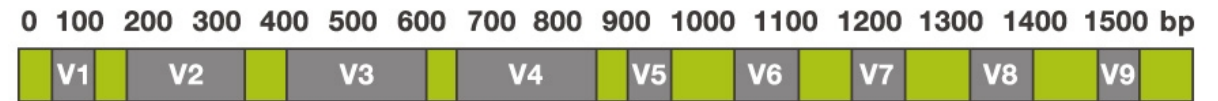
Ramazzotti and Bacci - 16S rRNA-Based Taxonomy Profiling in the Metagenomics Era - in [Metagenomics 2018](#)

Targeting 16S rRNA with PCR



16S rDNA is a perfect candidate because it is:

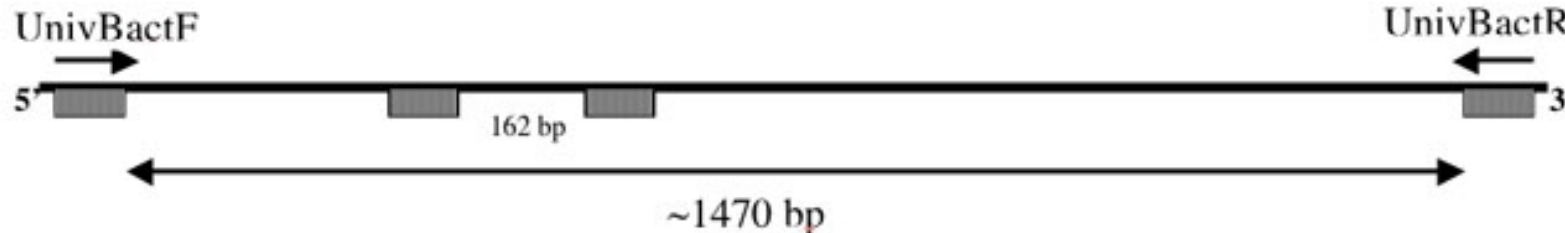
- present in all prokaryotic organisms
- sufficiently conserved in some regions
- highly variable in other regions



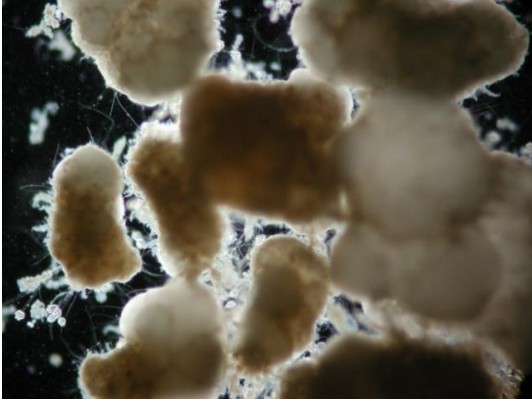
CONSERVED REGIONS: unspecific applications

VARIABLE REGIONS: group or species-specific applications

<u>Primer:</u>	<u>Primer Sequence:</u>	<u>Primer Length</u>	<u><i>E. coli</i> Position</u>
UnivBactF	5' GAG TTT GAT YMT GGC TC	17-mer	9 - 25
UnivBactR	5' GYT ACC TTG TTA CGA CTT	18-mer	1509 - 1492



A broad spectrum of environments



Aerobic granular sludges



Anammox granules

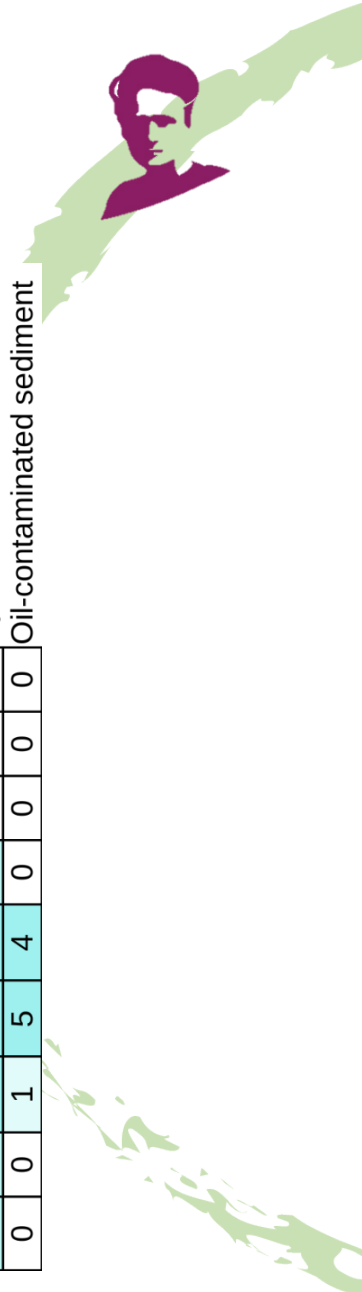


Anaerobic digestors



Activated sludges

Choice of the 16S region



EMBL-EBI | MGnify

MGnify

Number of studies @ EBI MGnify

fy

Biomes										
	V1	V2	V3	V4	V5	V6	V7	V8	V9	Biome
	117	99	261	419	155	47	52	42	67	Environmental
	112	94	236	388	145	45	49	38	63	Aquatic
	80	67	184	302	116	37	41	33	58	Marine
	78	72	133	214	47	24	24	36	26	Host-associated
	44	38	94	161	65	13	19	14	17	Sediment
	57	54	92	140	19	7	13	26	18	Digestive system
	51	48	58	94	21	10	12	18	13	Human
	8	8	37	64	7	2	5	11	7	Fecal
	11	11	31	59	6	3	7	14	11	Mammals
	16	13	29	54	12	4	5	3	2	Freshwater
	8	7	22	46	21	5	3	2	3	Oceanic
	3	3	23	45	6	1	2	6	4	Large intestine
	12	12	26	41	9	1	3	3	2	Engineered
	38	36	27	30	8	3	4	7	6	Oral
	7	8	24	31	10	1	2	3	7	Coastal
	4	4	24	29	9	1	2	3	2	Terrestrial
	7	7	13	24	5	1	4	4	3	Intertidal zone
	2	3	19	24	8	1	2	3	2	Soil
	1	2	12	18	10	6	2	1	1	Plants
	1	1	5	15	3	0	1	1	1	Lotic
	13	12	7	7	0	0	0	1	1	Saliva
	3	2	9	11	0	0	1	1	0	Birds
	0	0	0	11	1	0	0	0	1	Cold seeps
	6	6	11	7	2	2	3	2	0	Lentic
	1	1	5	11	3	3	1	1	1	Respiratory system
	3	3	5	11	1	0	0	0	0	Wetlands
	5	5	7	10	2	0	1	1	1	Wastewater
	1	1	3	9	3	1	0	1	0	Mixed
	1	1	4	8	1	0	0	0	0	Marginal Sea
	0	0	2	8	2	2	1	1	1	Nasopharyngeal
	2	2	6	8	5	1	1	1	1	Non-marine Saline
	1	0	2	8	4	0	0	0	0	Porifera
	3	1	2	7	2	1	1	0	0	Groundwater
	1	2	7	7	4	2	1	0	0	Rhizosphere
	0	0	3	7	3	0	0	0	0	Salt marsh
	1	1	2	6	1	0	0	0	0	Bioreactor
	4	4	4	6	0	0	0	0	1	Coral reef
	2	2	5	6	3	1	0	0	0	Estuary
	3	3	6	4	2	1	1	1	1	Fish
	3	3	4	6	2	0	0	0	0	Food production
	1	1	2	6	0	0	1	1	1	Gastrointestinal tract
	1	1	2	6	0	0	1	1	1	Reproductive system
	1	1	1	6	0	0	1	1	1	Vagina
	0	0	3	5	2	0	0	0	0	Abyssal plane
	5	4	1	1	0	0	0	0	0	Activated Sludge
	3	3	5	4	1	0	0	0	0	Deep subsurface
	1	1	2	5	2	0	0	0	0	Fermented beverages
	2	2	3	4	5	2	0	0	0	Hydrothermal vents
	0	0	1	5	4	0	0	0	0	Oil-contaminated sediment

Ramazzotti and Bacci - [Metagenomics 2018](#)

V3-V4 as general purpose target



all supposed to be "universal"

Name	Sequence (5'-3')	Reference	
Pro341F	CCTACGGGNBGCASCAG	Takahashi et al. ⁷	2014 most update V3V4 primer pair
Pro805R	GACTACNVGGGTATCTAATCC	Takahashi et al. ⁷	
314F (341F)	CCTACGGGNGGCWGCAG	Albertsen et al. ⁸	illumina default primer pair
805R	GACTACHVGGGTATCTAATCC	Albertsen et al. ⁸	
27F	AGAGTTTGATCCTGGCTCAG	Albertsen et al. ⁸	
534R	ATTACCGCGGCTGCTGG	Albertsen et al. ⁸	
515F	GTGCCAGCMGCCGCGGTAA	Albertsen et al. ⁸	
806R	GGACTACHVGGGTWTCTAAT	Albertsen et al. ⁸	

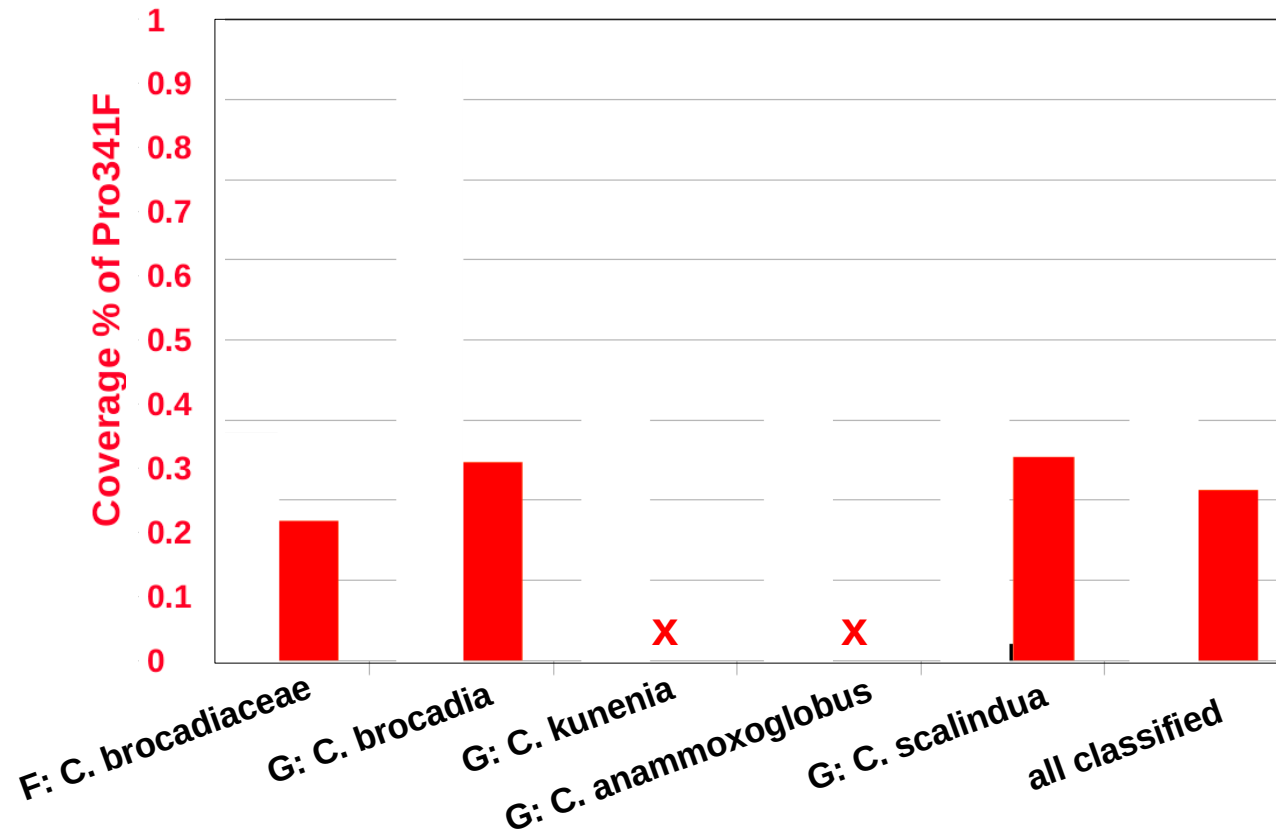


Computational screening to capture
expected coverage profiles of Takahashi et al. primers

Brocadiaceae – they were missing...



■ Pro341F



rootrank Root (0/1558788/12)
domain Bacteria (0/1502570/12)
phylum Planctomycetes (0/16957/12)
class Planctomycetacia (0/9720/12)
order Candidatus Brocadiales (0/395/12)
family Candidatus Brocadiaceae (0/395/12)
▼ genus Candidatus Brocadia (0/107/12)

↓
All 16S sequences of anammox species were aligned and studied to improve their coverage

Updating Takahashi with Illumina



Article | [Open Access](#) | [Published: 24 September 2020](#)

A novel universal primer pair for prokaryotes with improved performances for anammox containing communities

[Lorenzo Mazzoli](#), [Giulio Munz](#), [Tommaso Lotti](#) & [Matteo Ramazzotti](#) 

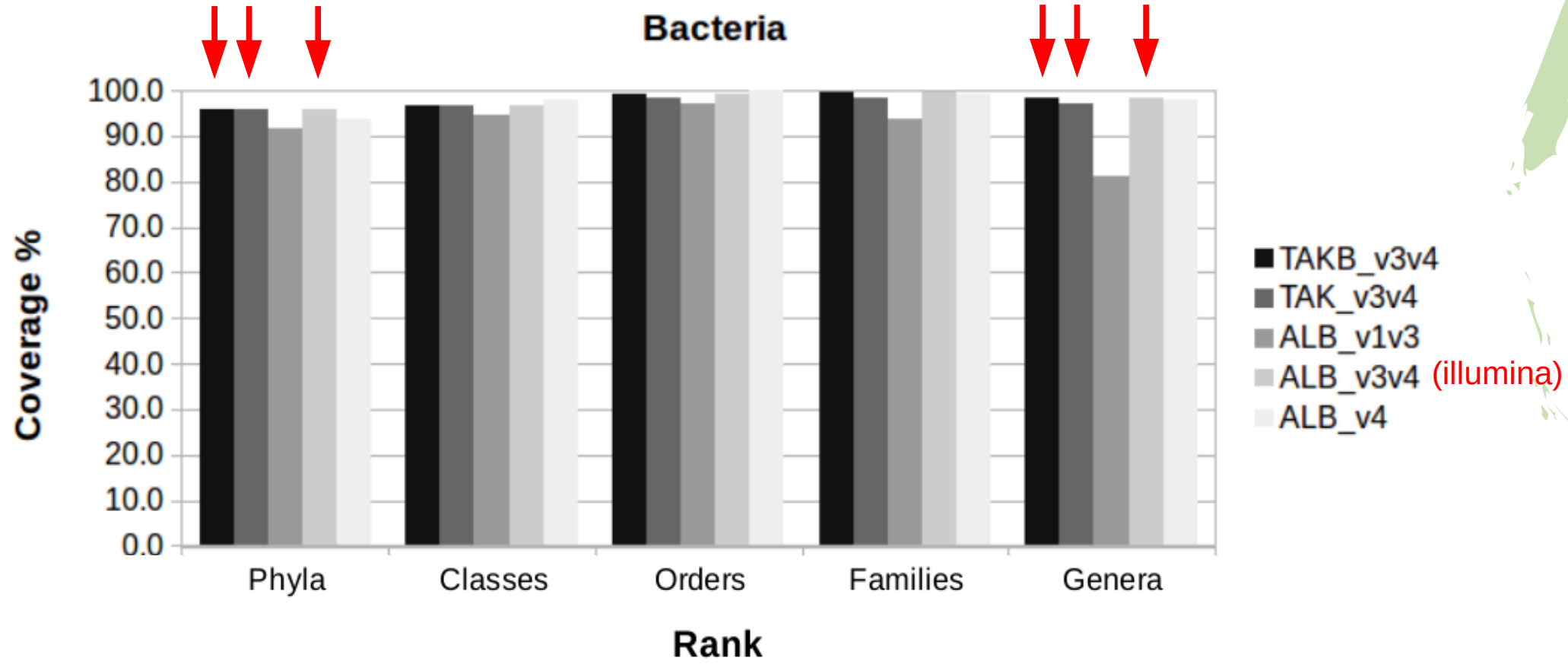
W = A or T

Name	Sequence (5'-3')	Reference
Pro341FB	CCTACGGGNNBGCW ^W SCAG	This work
Pro341F	CCTACGGGNNBGC ^W ASCAG	Takahashi et al. ⁷
Pro805R	GACTACNVGGGTATCTAATCC	Takahashi et al. ⁷
314F (341F)	CCTACGGGNGGC ^W WCAG	Albertsen et al. ⁸
805R	GACTACHVGGGTATCTAATCC	Albertsen et al. ⁸
27F	AGAGTTTGATCCTGGCTCAG	Albertsen et al. ⁸
534R	ATTACCGCGGCTGCTGG	Albertsen et al. ⁸
515F	GTGCCAGCMGCCGCGGTAA	Albertsen et al. ⁸
806R	GGACTACHVGGGTWTCTAAT	Albertsen et al. ⁸

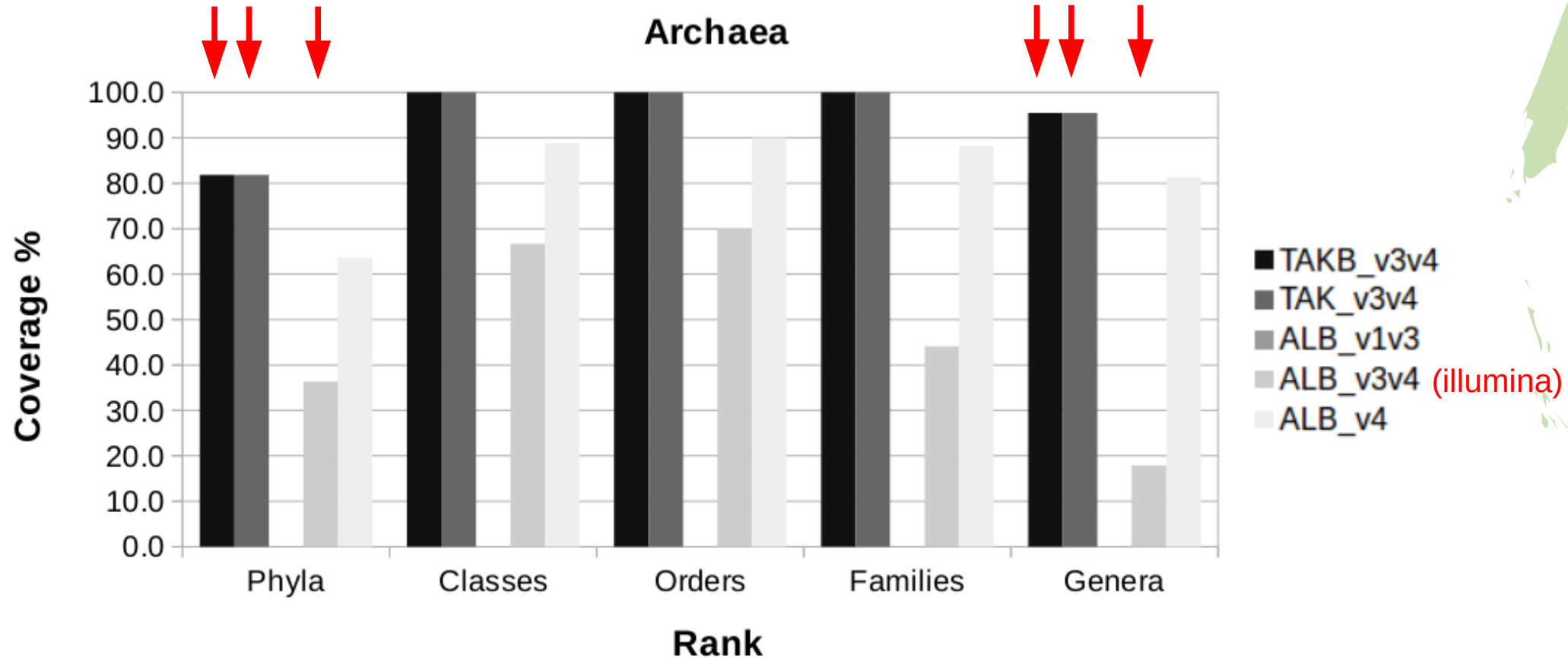
most update
V3V4 primer pair

Illumina broad range
V3V4 primer pair

Theoretical performances on bacteria



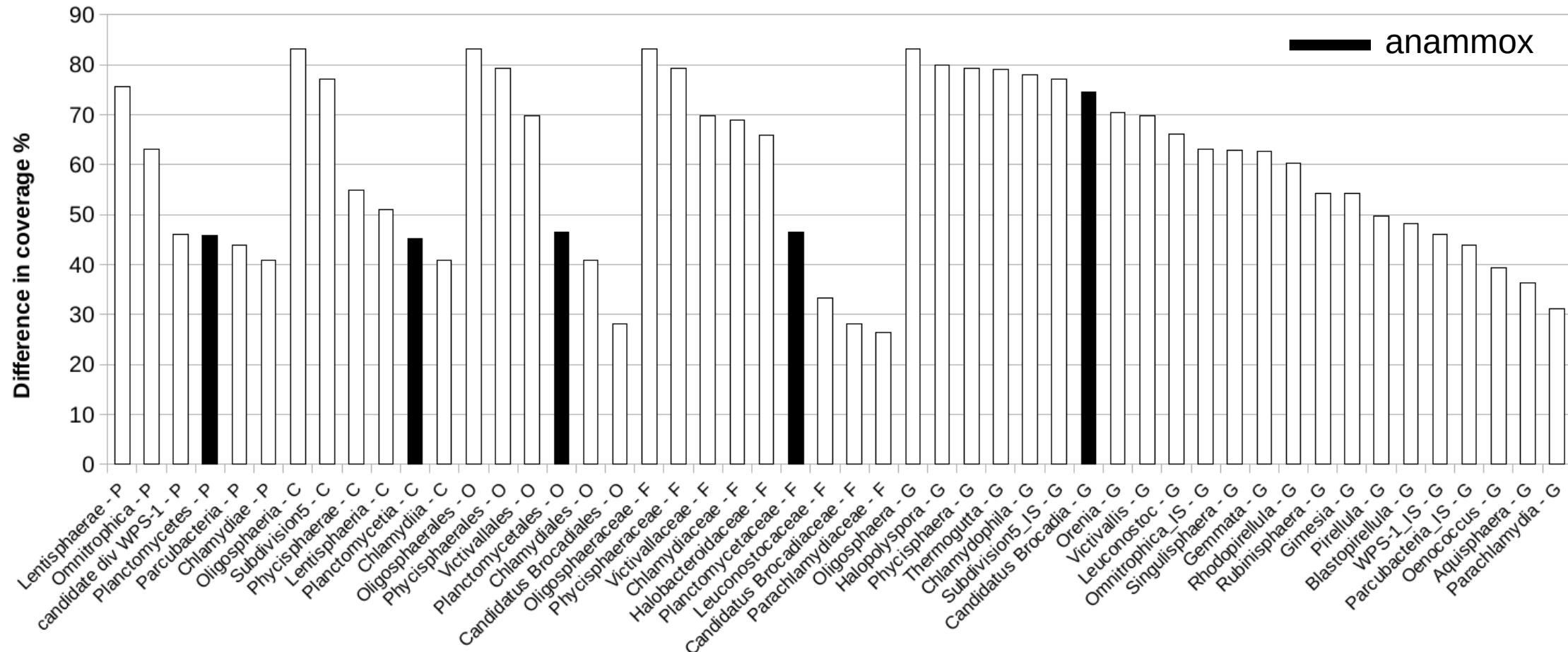
Theoretical performances on archaea



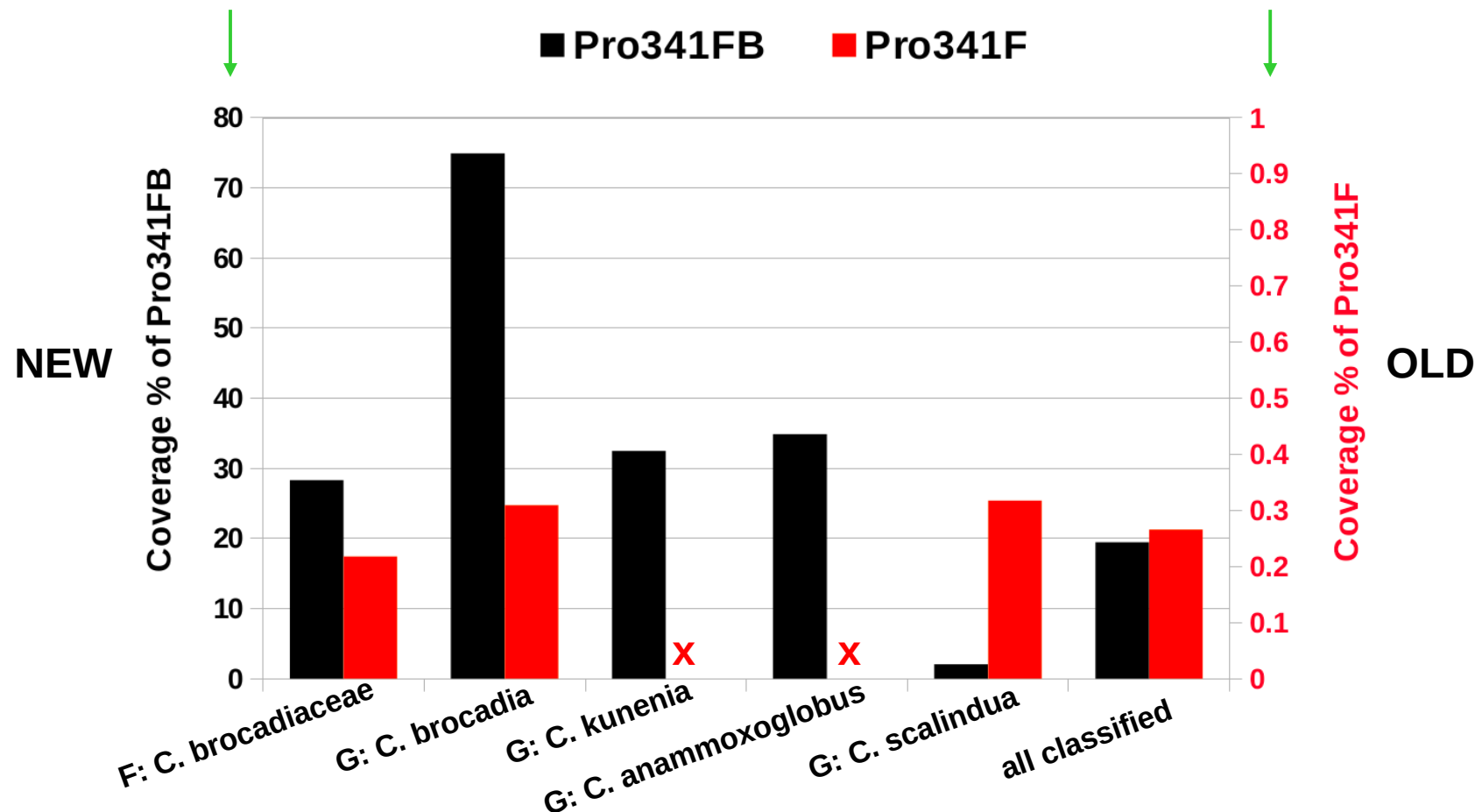
Theoretical performances on archaea



Ranks with more than 25% coverage increase



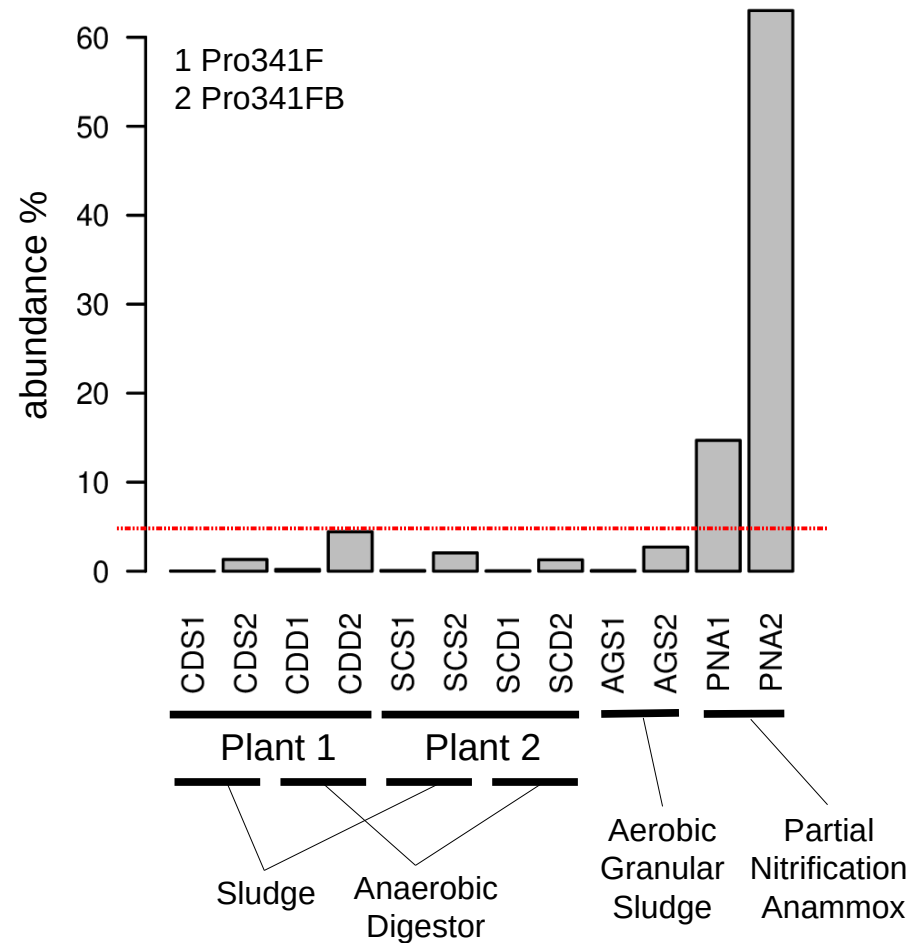
Theoretical performances on anammox



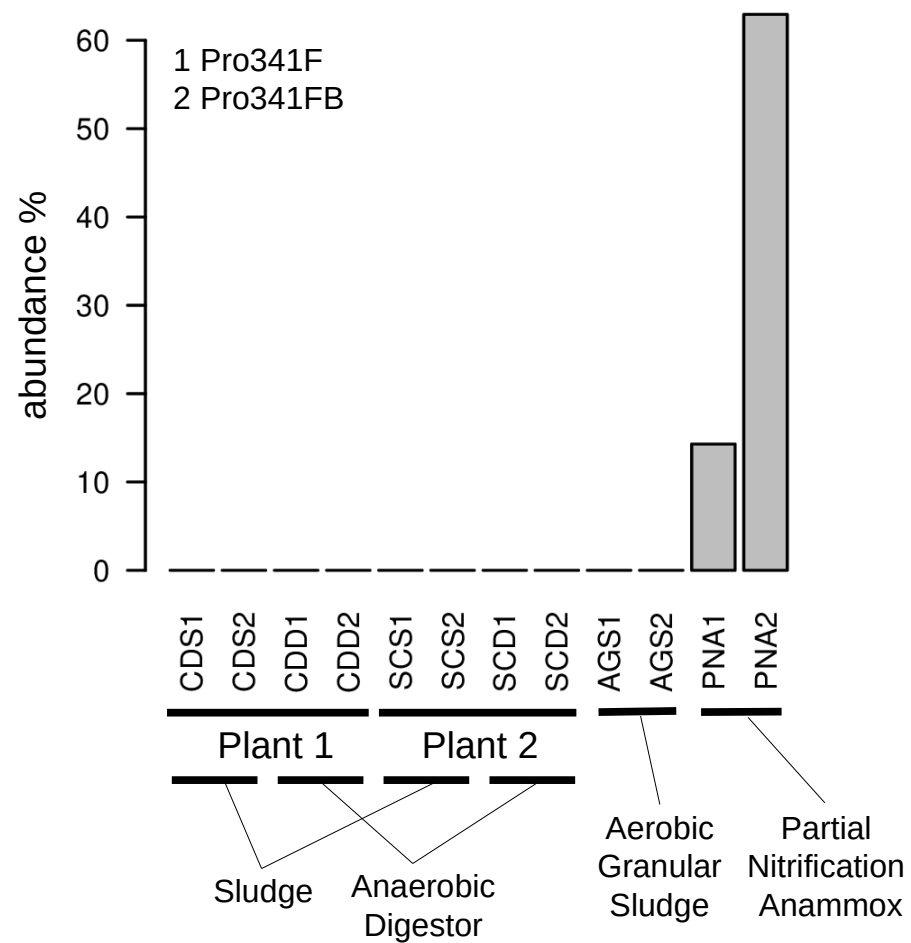
Efficacy of the new primer



Phylum Planctomycetes



Genus Candidatus Brocadia



Conclusions



- **Different environments may require different analytical strategies**
- **The PCR reaction is a competitive environment, primer degeneration alleviates competition**
- **Even one degeneration may seriously impact the interpretation of results**