Supplementary material for

Microbiome analysis and confocal microscopy of used kitchen sponges reveal massive colonization by *Acinetobacter*, *Moraxella* and *Chryseobacterium* species

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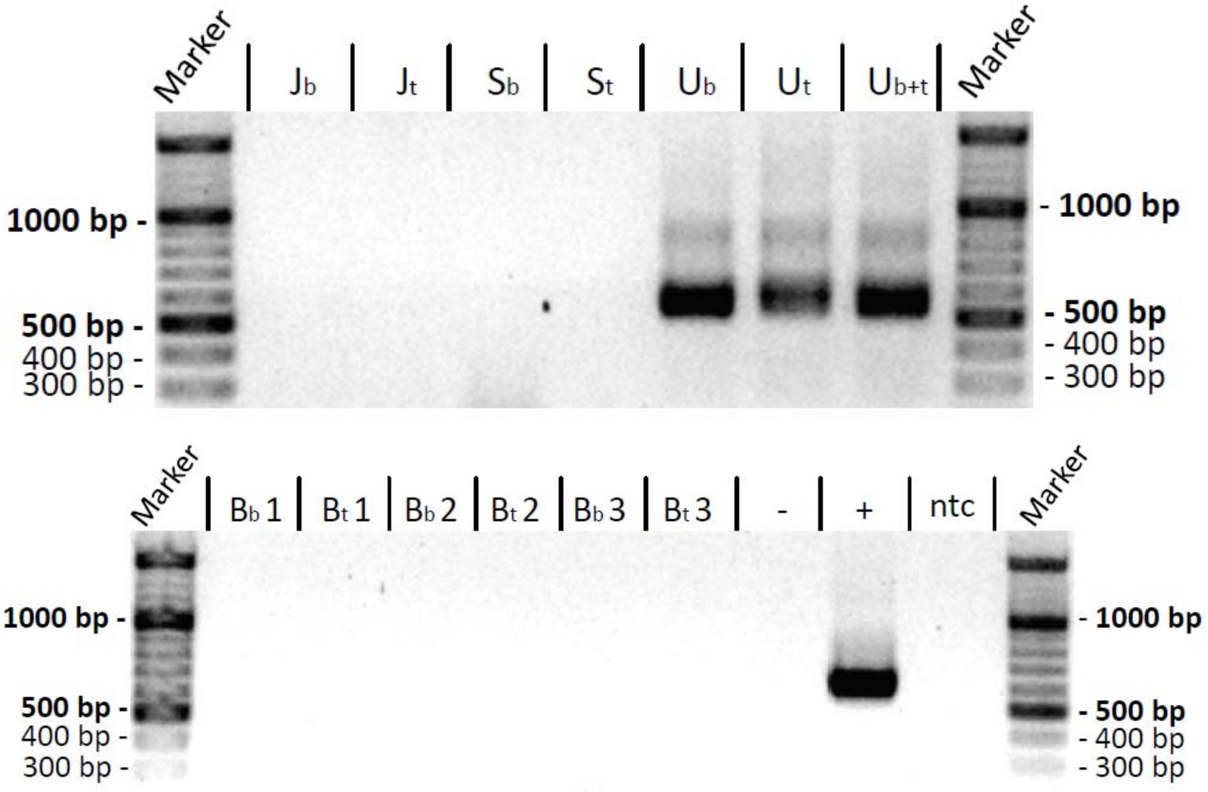

Figure S1. Results of a PCR assay targeting the16S rRNA gene in DNA extracts from five newly bought kitchen sponges (J, S, B; t = top, b = bottom). All new sponges did not yield PCR signals, while a used sponge sample (U) clearly did. Also a DNA extract from a negative extraction (-) yielded no PCR product. *E. coli* DNA was used a positive control. Ntc = no template control.

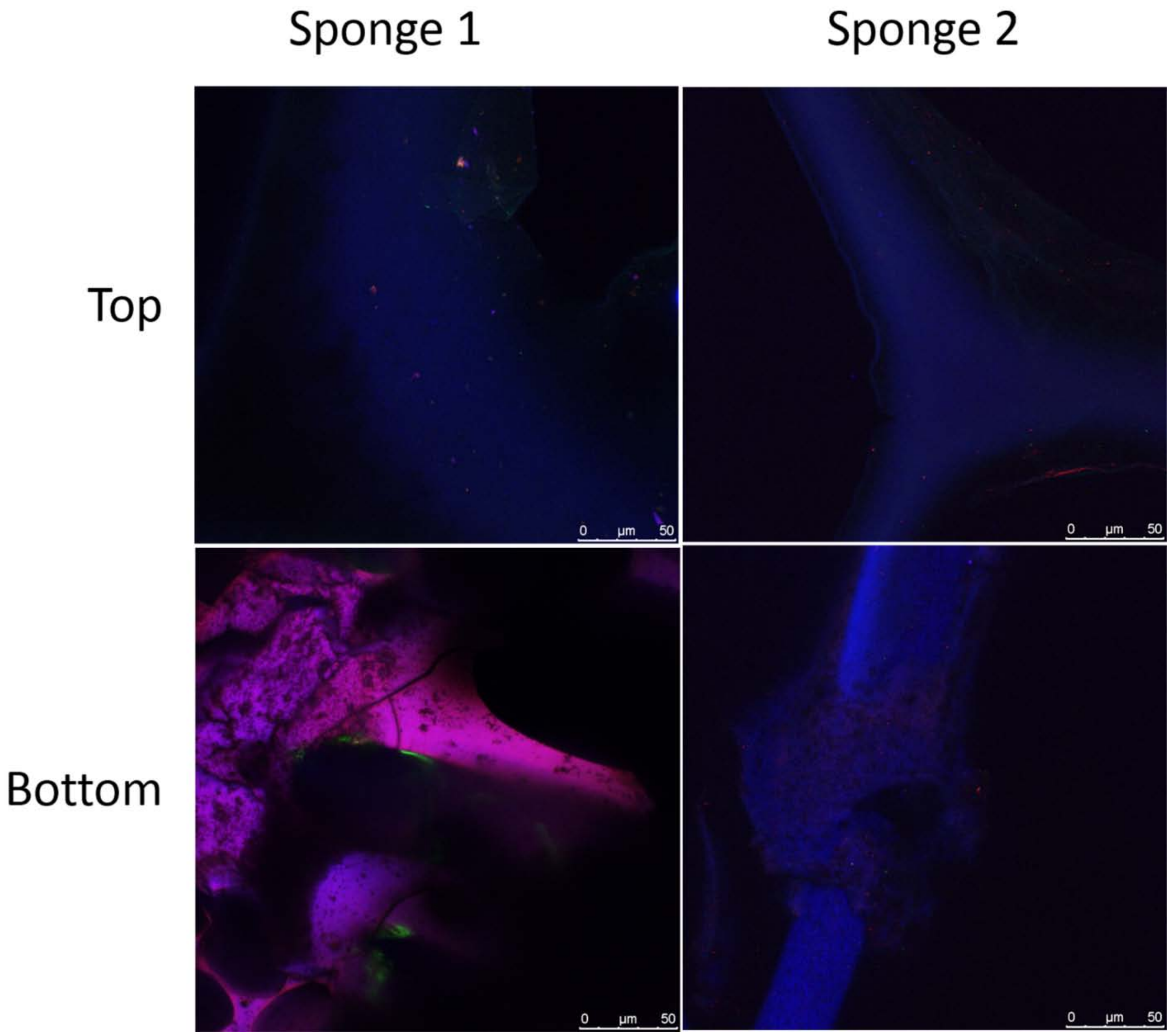

Figure S2 FISH–CLSM analysis of new kitchen sponges using the universal bacterial probe EUB338MIX labeled with Cy3 (expected signal: red) and the Gammaproteobacteria–specific probe Gam42a labelled with Cy5 (expected signal: green); no bacterial signals were detected. Blue= sponge autofluorescence.

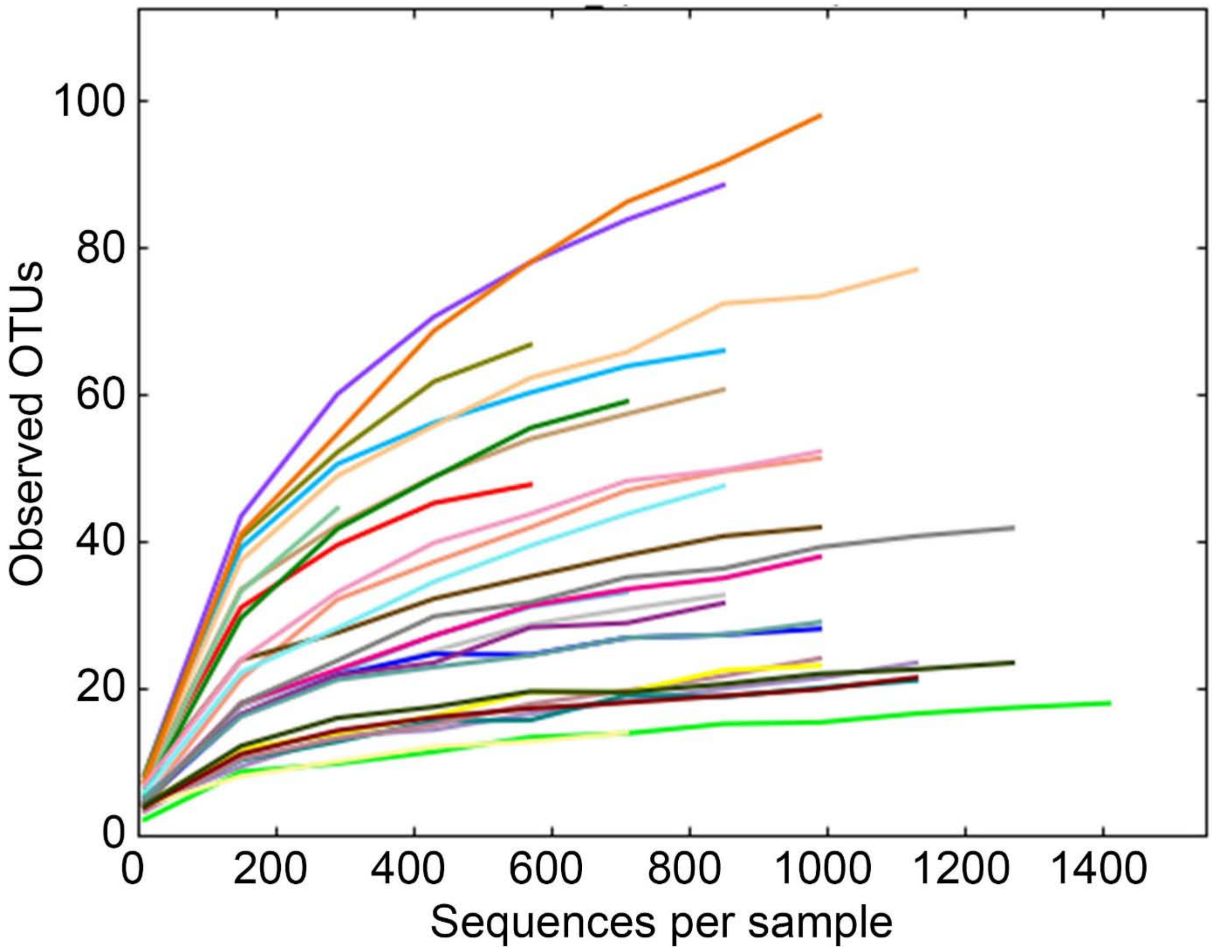

Figure S3 Rarefaction curves of the 28 sponge samples analyzed by pyrosequencing of the 16S rRNA amplicon libraries

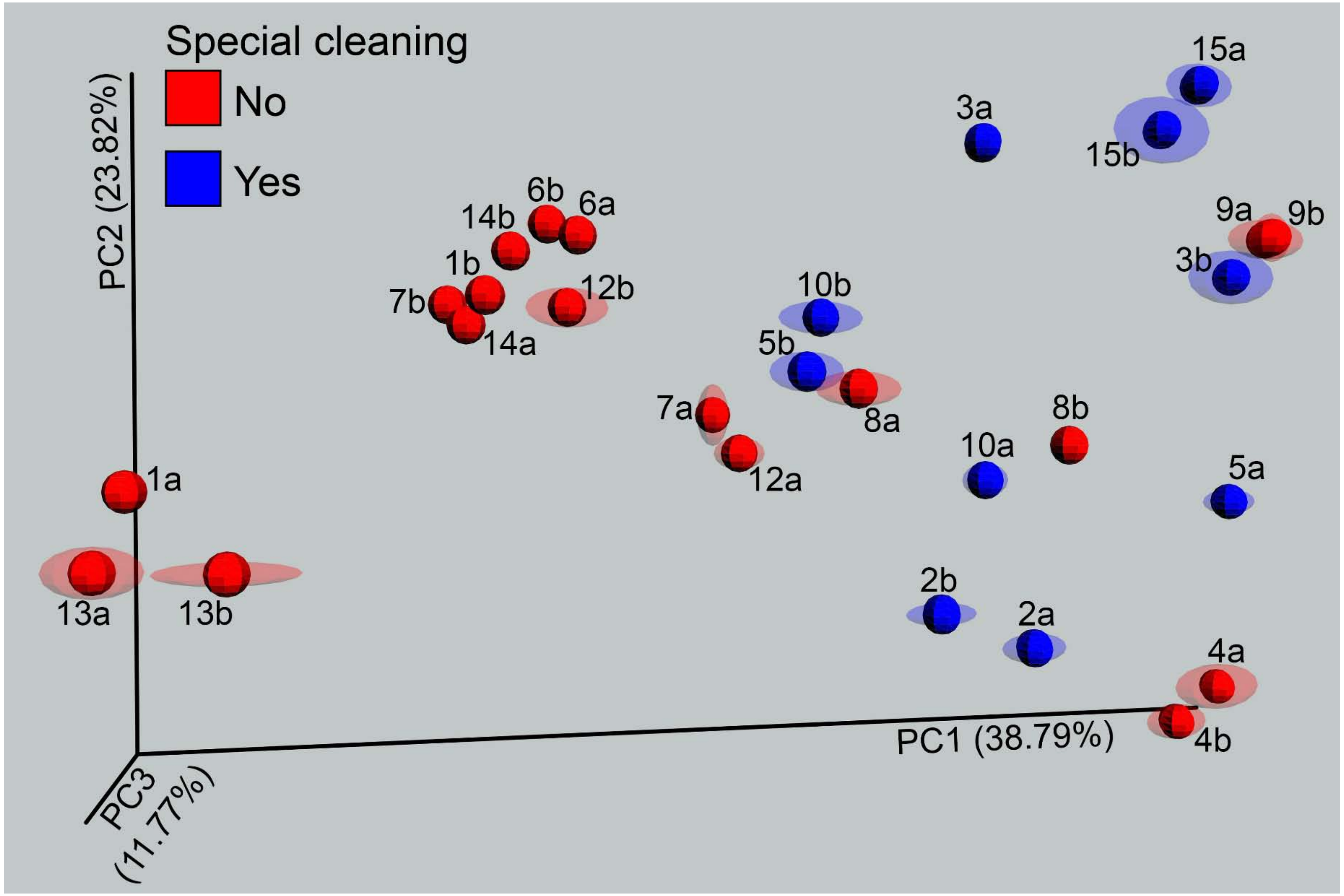

Figure S4 Beta–diversity plot of jacknifed weighted–Unifrac distances between sponge samples, colored by “special cleaning”, the only significant factor found (ADONIS, *p* = 0.022), and labeled by sample name (numbers indicate the individual sponges; letters indicate the sponge side, a= bottom, b= top)

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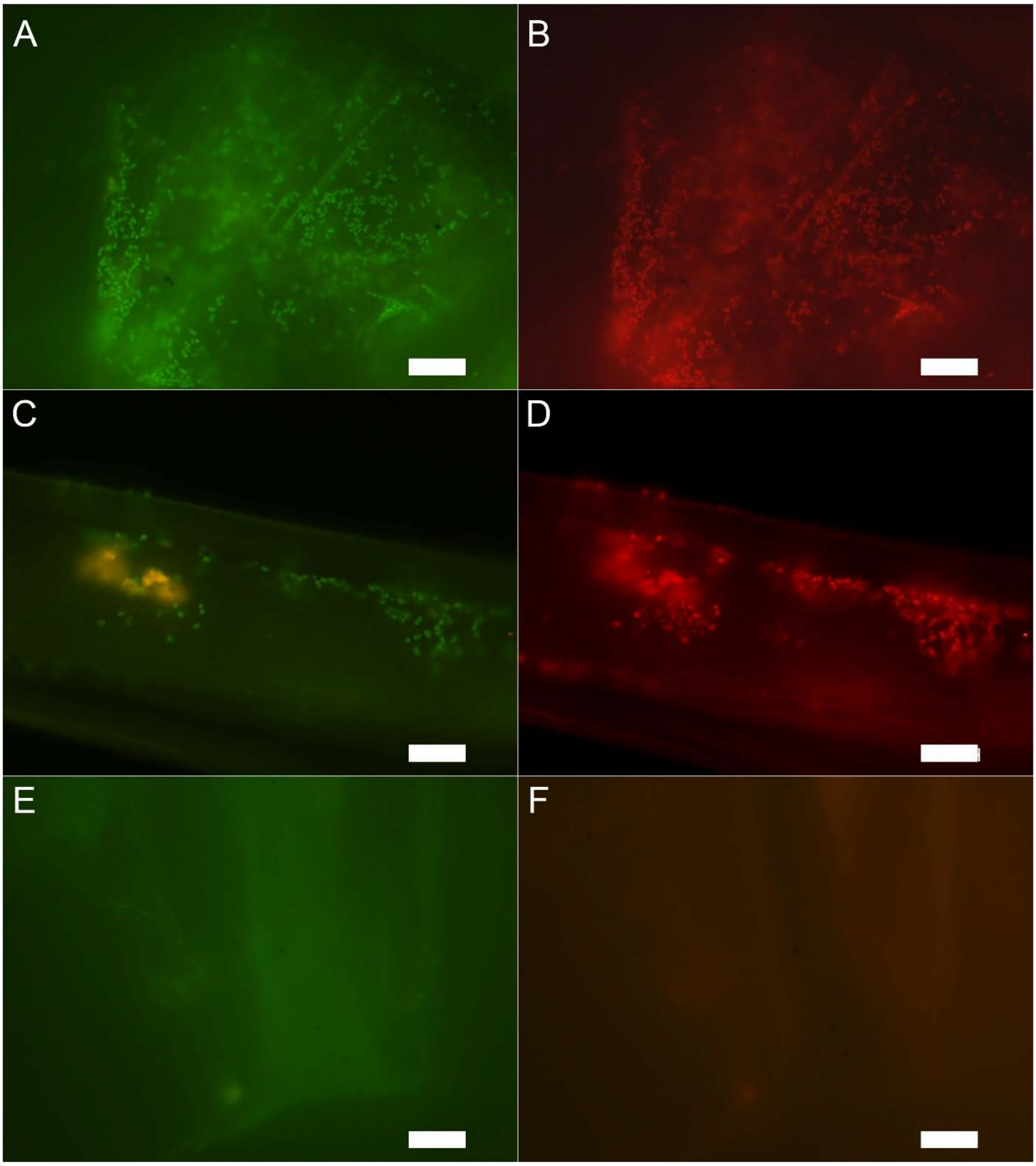

Figure S5 Examples of epifluorescent microscopy images used to assess the relative abundance of *Gammaproteobacteria* in selected FISH–stained sponge samples. A and C) Signal of FITC–labelled FISH probes Gam42a. B and D) Signal of the Cy3–labelled FISH probes EUB338MIX. E and F) FISH negative control, stained with the non–sense probe NONEUB labelled with FITC and Cy3, respectively. Scale bars: 15 µm.

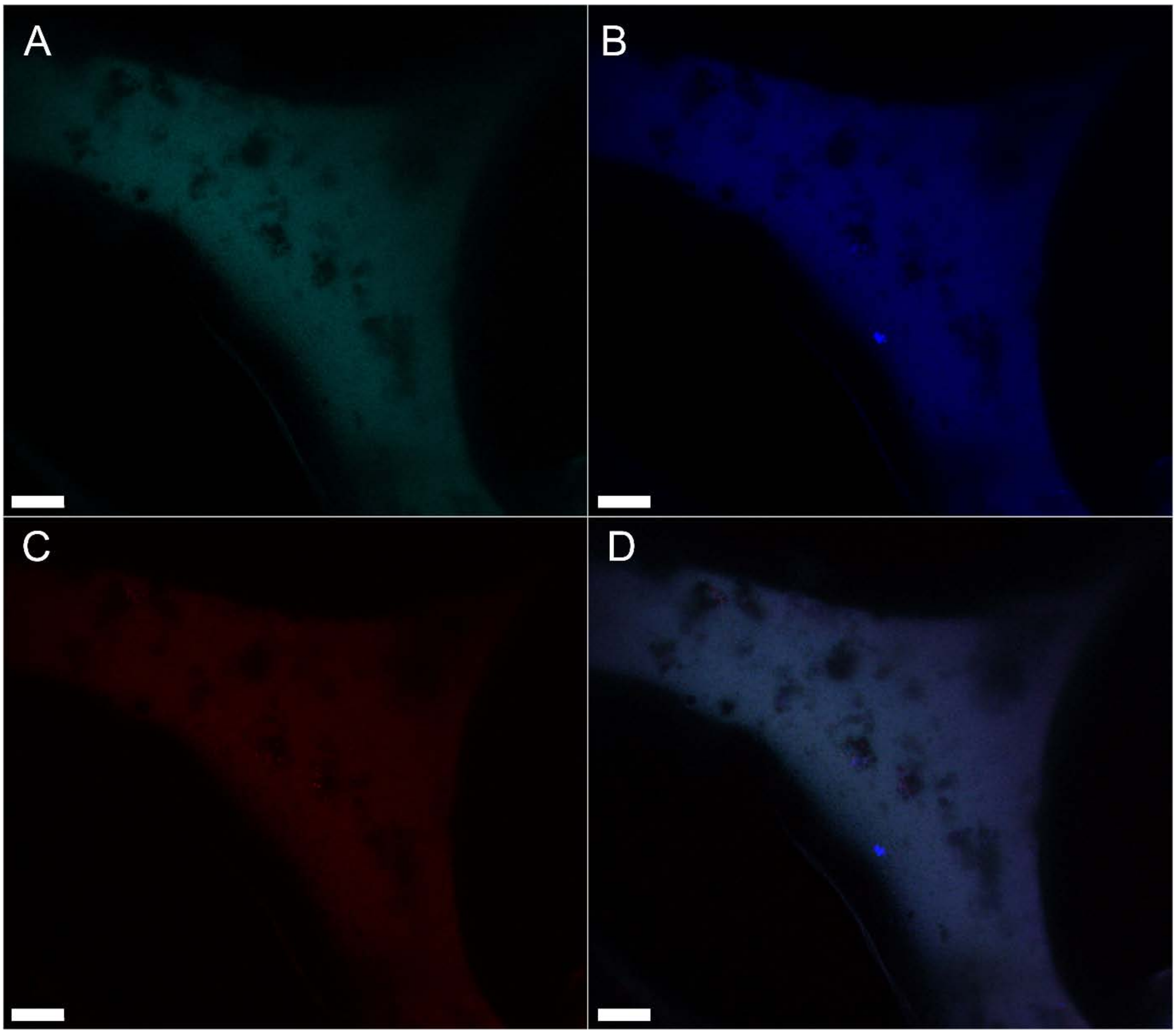

Figure S6 Maximum projection of a confocal laser–scanning microscopy image series, showing the FISH negative control of the kitchen sponge sample 6b. A) Sponge autofluorescence. B) Signal of FITC–labelled NONEUB probe. C) Signal of Cy3–labelled NONEUB probe. D) Overlap of A–C. Scale bars: 20 µm.

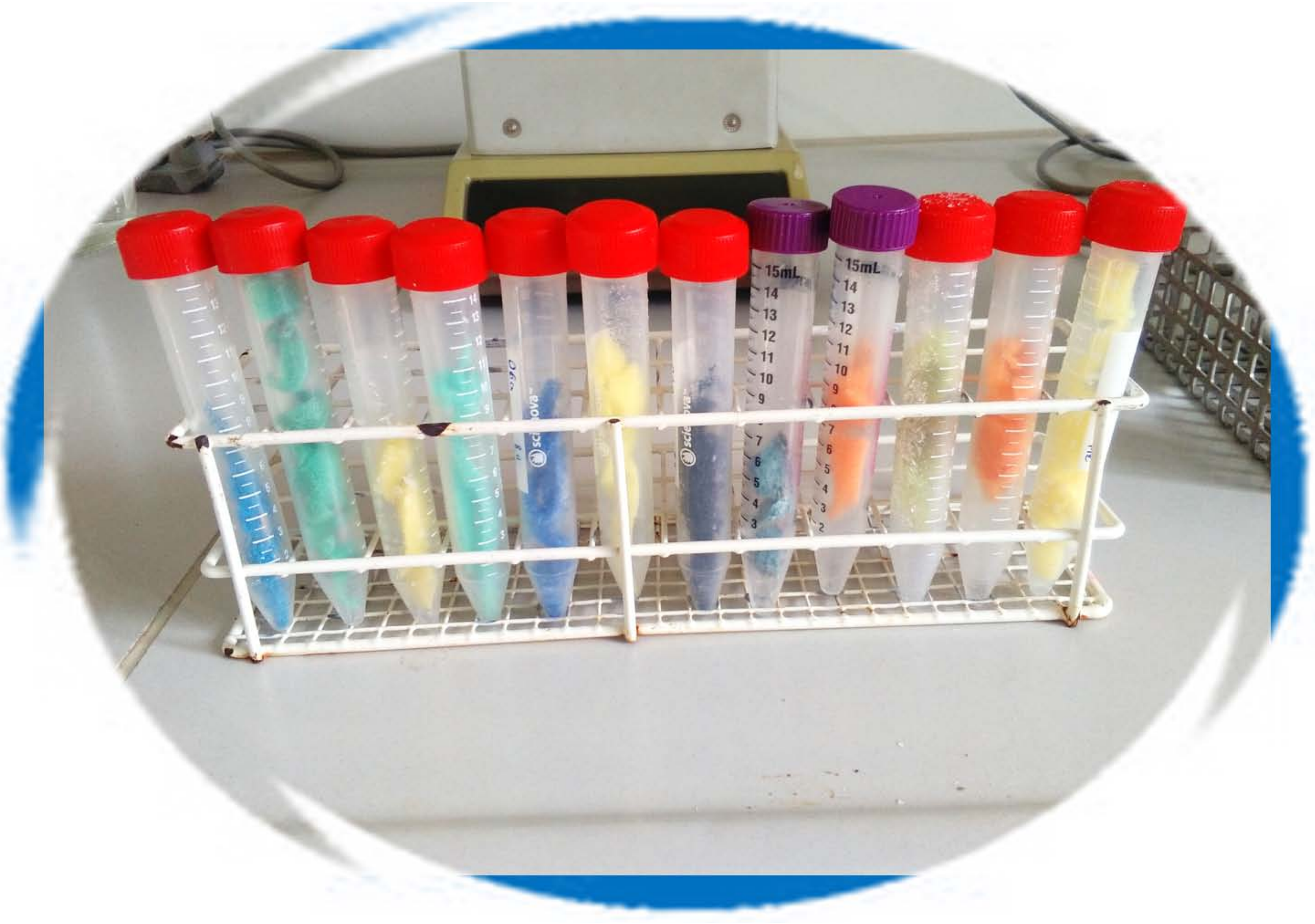

Figure S7 Examples of sponge samples used in this work

Table S1 Sponge samples analyzed in this work and their respective metadata

Sample	Intrinsic factors			Extrinsic factors			
	Sponge	Sponge side	Brand product	Change frequency	Use frequency	Special cleaning	Persons per household
1a	1	Bottom	No	Weekly	Not daily	No	1-3
2a	2	Bottom	No	Monthly	Not daily	Yes	1-3
3a	3	Bottom	Yes	Monthly	Daily	Yes	1-3
4a	4	Bottom	No	Weekly	Daily	No	5-20
5a	5	Bottom	No	Weekly	Daily	Yes	1-3
6a	6	Bottom	Yes	Monthly	Daily	No	1-3
7a	7	Bottom	No	Weekly	Not daily	No	1-3
8a	8	Bottom	No	Weekly	Not daily	No	1-3
9a	9	Bottom	Yes	Monthly	Daily	No	5-20
10a	10	Bottom	No	Monthly	Daily	Yes	1-3
12a	12	Bottom	No	Monthly	Daily	No	5-20
13a	13	Bottom	Yes	Monthly	Daily	No	1-3
14a	14	Bottom	No	Weekly	Not daily	No	1-3
15a	15	Bottom	No	Monthly	Daily	Yes	1-3
1b	1	Top	No	Weekly	Not daily	No	1-3
2b	2	Top	No	Monthly	Not daily	Yes	1-3
3b	3	Top	Yes	Monthly	Daily	Yes	1-3
4b	4	Top	No	Weekly	Daily	No	5-20
5b	5	Top	No	Weekly	Daily	Yes	1-3
6b	6	Top	Yes	Monthly	Daily	No	1-3
7b	7	Top	No	Weekly	Not daily	No	1-3
8b	8	Top	No	Weekly	Not daily	No	1-3
9b	9	Top	Yes	Monthly	Daily	No	5-20
10b	10	Top	No	Monthly	Daily	Yes	1-3
12b	12	Top	No	Monthly	Daily	No	5-20
13b	13	Top	Yes	Monthly	Daily	No	1-3
14b	14	Top	No	Weekly	Not daily	No	1-3
15b	15	Top	No	Monthly	Daily	Yes	1-3

Table S2 Alpha–diversity metrics of the used kitchen sponge samples analyzed in this work, as calculated from the rarefied pyrosequencing dataset at an even depth of 357 sequences per sample. Sample number indicate the individual sponge, while letters indicate the top (a) or bottom (b) site. The last row shows the significance of the factor "sponge" for each index (in red the significant values at $p < 0.05$).

Sample	Good's coverage	Shannon	Equitability	Dominance	Simpson	Simpson reciprocal[#]	Simpson evenness	PD whole tree	Chao1	Number of OTUs
1a	0.972	4.167	0.783	0.086	0.914	11.600	0.290	5.366	44.500	40
2a	0.927	4.385	0.739	0.112	0.888	8.927	0.146	7.253	88.083	61
3a	0.975	2.807	0.604	0.233	0.767	4.283	0.171	3.736	30.143	25
4a	0.944	3.402	0.669	0.146	0.854	6.830	0.201	5.241	97.333	34
5a	0.980	2.889	0.648	0.217	0.783	4.617	0.210	3.060	29.000	22
6a	0.975	2.499	0.532	0.365	0.635	2.742	0.105	4.301	32.000	26
7a	0.950	2.978	0.576	0.224	0.776	4.459	0.124	5.993	66.600	36
8a	0.958	4.565	0.801	0.067	0.933	14.996	0.288	7.457	59.000	52
9a	0.983	2.126	0.558	0.329	0.671	3.037	0.217	2.728	29.000	14
10a	0.969	2.947	0.627	0.197	0.803	5.077	0.195	3.485	37.000	26
12a	0.955	5.044	0.872	0.040	0.960	24.733	0.450	5.132	67.000	55
13a	0.944	4.772	0.815	0.060	0.940	16.563	0.286	7.465	77.000	58
14a	0.983	1.617	0.425	0.531	0.469	1.885	0.135	2.193	19.000	14
15a	0.994	1.879	0.508	0.448	0.552	2.233	0.172	2.657	13.250	13
1b	0.986	2.610	0.604	0.265	0.735	3.778	0.189	3.041	22.500	20
2b	0.947	3.865	0.712	0.133	0.867	7.509	0.175	5.632	71.500	43
3b	0.969	2.286	0.538	0.286	0.714	3.495	0.184	2.961	46.500	19
4b	0.964	3.548	0.692	0.147	0.853	6.790	0.194	5.661	46.143	35
5b	0.980	3.746	0.788	0.103	0.897	9.743	0.361	3.432	37.500	27
6b	0.986	0.927	0.251	0.749	0.251	1.334	0.103	2.513	15.000	13
7b	0.969	2.127	0.464	0.430	0.570	2.327	0.097	3.112	33.167	24
8b	0.952	4.092	0.737	0.110	0.890	9.072	0.193	6.561	64.000	47
9b	0.997	1.638	0.546	0.412	0.588	2.425	0.303	1.918	8.000	8
10b	0.972	2.618	0.551	0.325	0.675	3.074	0.114	3.857	38.250	27

12b	0.955	4.172	0.764	0.098	0.902	10.185	0.231	4.406	57.333	44
13b	0.927	5.067	0.851	0.042	0.958	23.791	0.384	5.867	102.625	62
14b	0.983	2.387	0.627	0.238	0.762	4.201	0.300	1.924	29.000	14
15b	0.989	2.250	0.550	0.378	0.622	2.644	0.156	2.892	18.500	17
ANOVA (between sponges), *p* value	< 0.001	< 0.001	0.005	0.012	0.012	0.045[##]	0.08	0.001	0.003	< 0.001

[#] Not normally distributed variable

[##] Kruskall–Wallis test

Table S3 Comparison of the relative abundance of major taxa (family level) between our work and the sponge sample analyzed by Flores *et al.*, 2013. Mean (± Std error for this work's samples) is indicated.

Family	Relative abundance (% of microbiome)	
	This work	Flores *et al*., 2013*
Moraxellaceae	36.04 ± 4.86	40.39
Pseudomonadaceae	9.58 ± 3.55	7.48
Rhizobiaceae	4.01 ± 0.78	3.22
Flavobacteriaceae	1.31 ± 0.76	1.98
Staphylococcaceae	0.037 ± 0.022	0.02
[*Weeksellaceae*]	21.90 ± 3.70	n.d.**
Sphingomonadaceae	3.12 ± 1.02	0.42
Xanthomonadaceae	3.21 ± 1.25	16.16
Enterobacteriaceae	1.18 ± 0.24	16.86
Acetobacteraceae	0.18 ± 0.09	4.40

*Flores, G. E., Bates, S. T., Caporaso, J. G., Lauber, C. L., Leff, J. W., Knight, R. & Fierer, N. Diversity, distribution and sources of bacteria in residential kitchens. *Env Microbiol* **15**, 588–596 (2013)
**n.d. = not detected

Table S4 Properties of the FISH probes used in this work

Probe name	Sequence (5'–3')	Fluorochrome	Target	% Formamide (at 42°C)
EUB338*	GCTGCCTCCCGTAGGAGT	Cy3	Most bacteria	35
EUB338II*	GCAGCCACCCGTAGGTGT	Cy3	*Planctomycetales*	35
EUB338III*	GCTGCCACCCGTAGGTGT	Cy3	*Verrucomicrobiales*	35
Gam42a**	GCCTTCCCACATCGTTT	FITC	*Gammaproteobacteria*	35
Gam42a–competitor (unlabelled Bet42a probe) **	GCCTTCCCACTTCGTTT	/	*Betaproteobacteria*	35
NONEUB***	ACTCCTACGGGAGGCAGC	Cy3	/	35
NONEUB***	ACTCCTACGGGAGGCAGC	FITC	/	35

*, ** and *** used mixed in equimolar concentration

Log file S1 Results of length/quality filtering of the pyrosequencing reads, obtained with QIIME 1.9.

```
Number raw input seqs          223741

Length outside bounds of 480 and 540             136675
Num ambiguous bases exceeds limit of 6           197
Missing Qual Score                               0
Mean qual score below minimum of 25              5245
Max homopolymer run exceeds limit of 6           363
Num mismatches in primer exceeds limit of 1:     46882

Sequence length details for all sequences passing quality filters:
Raw len min/max/avg            480.0/540.0/516.7
Wrote len min/max/avg          450.0/510.0/486.7

Barcodes corrected/not         281/1198
Uncorrected barcodes will not be written to the output fasta file.
Corrected barcodes will be written with the appropriate barcode category.
Corrected but unassigned sequences will not be written unless --retain_unassigned_reads is
enabled.

Total valid barcodes that are not in mapping file     0
Sequences associated with valid barcodes that are not in the mapping file will not be written.

Barcodes in mapping file
Num Samples 28
Sample ct min/max/mean:        386 / 1520 / 1185.04
Sample         Sequence Count         Barcode
15b            1520           AGTACGCTAT
6b             1453           TAGAGACGAG
4b             1417           TACGAGTATG
2a             1402           ACGCTCGACA
13b            1385           AGACTATACT
15a            1383           TCTACGTAGC
9a             1379           TAGTATCAGC
6a             1375           ATATCGCGAG
5b             1351           TACTCTCGTG
1b             1347           CGTGTCTCTA
12b            1275           ACTGTACAGT
12a            1256           ATACGACGTA
14a            1249           CGTCTAGTAC
3a             1230           AGACGCACTC
8a             1222           ACGACTACAG
5a             1216           ATCAGACACG
14b            1206           AGCGTCGTCT
10a            1108           TCTCTATGCG
7a             1098           TGTACTACTC
3b             1098           CGTAGACTAG
4a             1095           AGCACTGTAG
10b            1082           ACTACTATGT
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2b	1028	CTCGCGTGTC
1a	1008	ACGAGTGCGT
13a	944	TCACGTACTA
9b	903	ACGCGAGTAT
7b	765	TCGTCGCTCG
8b	386	ACATACGCGT
Total number seqs written		33181

Video file S1: Video animation of the sponge sample 9B. The sponge sample was stained by FISH, using the Cy3–labelled EUB338MIX probe, and observed by confocal microscopy; this confocal series is composed of 52 optical slices and was acquired using a Z–step of 0.63µm. The three–dimensional model was created with the software Imaris 8.3 (Bitplane, Zurich, Switzerland), by converting the sponge autofluorescence signals into iso–surfaces and the bacterial FISH–conferred signals into spheres, respectively.

Partial 16S rRNA gene sequences of the OTUs displayed in Fig. 2 of the main text.

Not aligned

>OTU586 (Sphingobium)
AACGAACGCTGGCGGCATGCCTAATACATGCAAGTCGAACGAGATCTTCGGATCTA
GTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCTTGGGTTCGGAATAACTTCT
GGAAACGGAAGCTAATACCGGATGATGACGTAAGTCCAAAGATTTATCGCCCAAG
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ATCCTTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCA
GACTCCTACGGGAGGCAGCAGTAGGGAATATTGGACAATGGGCGAAAGCCTGATC
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GGTAATATAGAGACACG
>OTU2089 (Agrobacterium)
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GAAACTGGAATTAATACCGCATACGCCCTACGGGGGAAAGATTTATCGGGGAAGG
ATTGGCCCGCGTTGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGAT
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GCCATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTCACCGATGAA
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GTAATACTGTAGTCGT
>OTU1492 (Acinetobacter)
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GGGGACAACATTTCGAAAGGAATGCTAATACCGCATACGTCCTACGGGAGAAAGC
AGGGGATCTTCGGACCTTGCGCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGG
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TGGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTTTAGTAATACCTAGAGATAG
TGGACGTTACTCGCAGAATAAGCACCGGCTAACTCCGTAGCCG
>OTU47 (Chryseobacterium)
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GGGATAGCCTTTCGAAAGGAAGATTAATACCCCATAATATAATGATTGGCATCAAT
TATTATTGAAAACTCCGGTGGATAGAGATGGGCACGCGCAAGATTAGATAGTTGGT
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GGATTGTAAACTTCTTTTGTACAGGGATAAACCTACTCTCGTGAGGGTAGCTGAAG
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>OTU2007 (Pseudomonas)
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>OTU99 (Moraxella)
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>OTU3112 (Chryseobacterium)
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>OTU1844 (Brevundimonas)
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>Moraxella osloensis DSM 6998 (NR113392)
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CACACCATGGGAGTCTATTGCACCAGAAGTAGGTAGCCTAACGAAAGAGGGCGCT
TACCACGGTGTGGTCGATGACTGGGGTGAAGTCGTAACAAGGTAGCC
>Moraxella osloensis A1920 (NR104936)
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