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Supplemental Information for:

Spatial structure in the "Plastisphere": Molecular resources for imaging microscopic communities on plastic marine debris

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Table S1: *In silico* tests of probe coverage and specificity. For each oligonuclotide probe designed, the number of sequences of the target taxon in ARB Silva release 132 is shown (# targets), as well as the number (# exact match) and fraction (coverage of target) of these that exactly matched the probe. The number of non-target sequences with 0 or 1 mismatches to the probe (non-target hits) are also shown, as are the mathFISH predictions of hybridization efficiency in 20% formamide and of the free energy of the hybridization reaction ($\Delta G_{o\ overall}$). Calculations were performed for 12 to 15 target sequences per probe and the range of resulting values is shown. The two Vib probes and Fla891 showed mismatches with families outside their target clade, but most of these families were not abundant on plastic samples.

Probe	target taxon (Sequence 5'-3')	# targets	# exact match	Coverage of target	Non-target hits	hybridization efficiency	$\Delta G_{o\ overall}$ (kcal/mol)
Rho1682	*Rhodobacteraceae* CCTTCTCGCGAACTTACGGAGG	513	437	85%	9	100%	-19 - -24
Rho420	*Rhodobacteraceae* TCAGTAAGGAGTACTTAGCCTTCG	513	277	54%	0	99%	-13 - -17
Bac1058	*Bacteroidetes* TGAATGGCTGCTTCCAAGCCAACA	3065	2535	83%	0	99.7%	-15 - -16
Alt811	*Alteromonadaceae* ACAGCTAGTAGACAGCGTTTACG	1091	935	82%	0	99%	-15 - -18
Vib2300	*Vibrionaceae* TAACCTCACGATGTCCAACCGTG	2242	1906	85%	800 Pasteurellales, 20 Oceanospirillales	100%	-14 - -16
Vib 1759	*Vibrionaceae* AGCCACCTGGTATCTGCGACT	2242	2223	99%	300 Shewanellaceae, 8 Alteromonadaceae	100%	-14 - -15
Fla891	*Flavobacteriaceae* AGTTTGTCAGGAATTGGTAGGCG	864	772	89%	100 Rikenenellaceae (gut bacteria)	99%	-15 - -19
Act1894	*Actinobacteria* AGTTACCACCGCCGTTTACTGG	9326	8417	90%	2	100%	-12 - -18

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Table S2: Taxonomy of pure cultures on which we carried out probe validation. Probe oligonucleotide name ("tested probes" column, top) and attached fluorophore ("tested probes" column, bottom) were tested on 55 different cultures. We tested single probes on single cultures and a set of 7 probes on single cultures. Some cultures were isolated from plastic marine debris (PMD). PE: Polyethylene; Plas.: unidentified plastic; PHA: Polyhydroxyalkanoate; PS: Polystyrene; PP: Polypropylene; PLA: Polylactic acid. Green indicates that probes hybridized with bacterial cells and were visible under the microscope as expected (true positive). Red indicates that probes did not hybridize with non-target cells (true negative) as expected. Blue indicates that the probe did not react as we expected and showed a false positive or false negative result.

ID	from PMD	Phylum	Class	Family	Genus	Tested probes								
A257		Actino-bacteria	Actino-bacteria	Micrococca-ceae	*Micrococcus*	Eub338 DY415	Gam42a DY505	Alf968 Atto620		Act1894 DY490			Vib1749 TRX	
G181	PE	Actino-bacteria	Micro-coccales	Micrococca-ceae	*Micrococcus* sp.	Eub338 DY415	Gam42a DY505	Alf968 Atto620		Act1894 DY490				
A106		Actino-bacteria	Nocardi-oidaceae	Nocardioi-daceae	*Nocardioides*	Eub338 DY415	Gam42a DY505	Alf968 Atto620		Act1894 DY490	Fla891 DY490	Vib2300 TRX		
B016		Bacter-oidetes	Cyto-phagia	Flammeo-virgaceae	*Marivirga*	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Gam42a Atto647N	Vib1749 TRX	Gam42a CY5
A861	Plas.	Bacter-oidetes	Cyto-phagia	Flammeo-virgaceae	*Porifericola*	Eub338 DY415	Gam42a DY505	Alf968 Atto620		Bac1058 RRX				
G125		Bacter-oidetes	Cyto-phagia	Flavobac-teriaceae	*Cellulophaga* sp.	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Vib2300 TRX	Vib1749 TRX	Fla891 DY490
G057	PHA	Bacter-oidetes	Flavo-bacteriia	Flavobac-teriaceae	*Polaribacter* sp.	Eub338 DY415					Fla891 DY490			
A739	Plas.	Bacter-oidetes	Flavo-bacteriia	Flavobac-teriaceae	*Tenacibaculum*	Eub338 DY415	Gam42a DY505	Alf968 Atto620			Fla891 DY490			
G033	PS	Bacter-oidetes	Flavo-bacteriia	Flavobac-teriaceae	*Tenacibaculum* sp.	Eub338 DY415	Gam42a DY505	Alf968 Atto620			Fla891 DY490			
		Cyano-bacteria	Cyano-phyceae	Nostoca-ceae	*Anabaena*	Eub338 DY415	Gam42a DY505	Alf968 Atto620						
A403		Proteo-bacteria	Alpha-proteob.	Erythrobac-teraceae	*Alterythrobacter*	Eub338 DY415	Gam42a DY505	Alf968 Atto620						
A015		Proteo-bacteria	Alpha-proteob.	Erythrobac-teraceae	*Erythrobacter*	Eub338 DY415	Gam42a Atto647N	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505		Vib1749 TRX	Gam42a CY5
A788	Plas.	Proteo-bacteria	Alpha-proteob.	Erythrobac-teraceae	*Erythrobacter*	Eub338 DY415	Gam42a DY505	Alf968 Atto620						
B074		Proteo-bacteria	Alpha-proteob	Hyphomo-nadaceae	*Hyphomonas*	Eub338 DY415	Gam42a DY505	Alf968 Atto620						

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ID	from PMD	Phylum	Class	Family	Genus	Tested probes								
B089		Proteo-bacteria	Alpha-proteob.	Hyphomo-nadaceae	*Hyphomonas*	Eub338 DY415	Gam42a CY5	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505		Vib1749 TRX	
A860	Plas.	Proteo-bacteria	Alpha-proteob.	Phyllobac-teriaceae	*Nitratirductor*	Eub338 DY415	Gam42a DY505	Alf968 Atto620						
A328		Proteo-bacteria	Alpha-proteob.	Rhodobac-teraceae	*Ahrensia*	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho420 DY490					
A067		Proteo-bacteria	Alpha-proteob.	Rhodobac-teraceae	*Paracoccus*	Eub338 DY415				Bac1058 RRX				
A100		Proteo-bacteria	Alpha-proteob.	Rhodobac-teraceae	*Paracoccus*	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Vib2300 TRX	Vib1749 TRX	Gam42a CY5
A303		Proteo-bacteria	Alpha-proteob.	Rhodobac-teraceae	*Pseudorhodobacter*	Eub338 DY415			Rho1682 DY490					
A309		Proteo-bacteria	Alpha-proteob.	Rhodobac-teraceae	*Pseudorhodobacter*	Eub338 DY415			Rho420 DY490					
A329		Proteo-bacteria	Alpha-proteob.	Rhodobac-teraceae	*Sulfitobacter*	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490					
B144		Proteo-bacteria	Alpha-proteob.	Rhodobac-teraceae	*Sulfitobacter*	Eub338 DY415	Gam42a Atto647N	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Rho420 DY490	Vib1749 TRX	Gam42a CY5
A382		Proteo-bacteria	Alpha-proteob.	Sphingomo-nadaceae	*Sphingomonas*	Eub338 DY415	Gam42a DY505	Alf968 Atto620						
A365		Proteo-bacteria	Alpha-proteob.	Sphingomo-nadaceae	*Sphingopyxis*	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Vib2300 TRX		
G058	PHA	Proteo-bacteria	Gamma-proteob.	Alteromona-daceae	*Alteromonas*	Eub338 DY415					Alt811 DY505	Vib2300 TRX	Vib1749 TRX	
G021	PP	Proteo-bacteria	Gamma-proteob.	Alteromona-daceae	*Alteromonas marina*	Eub338 DY415	Gam42a CY5	Gam42a DY647P1			Alt811 RRX			
G041	PHA	Proteo-bacteria	Gamma-proteob.	Alteromona-daceae	*Alteromonas sp.*	Eub338 DY415	Gam42a Atto647N	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505		Vib1749 TRX	Gam42a CY5
G069	PP	Proteo-bacteria	Gamma-proteob.	Alteromona-daceae	*Alteromonas sp.*	Eub338 DY415	Gam42a CY5	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505		Vib1749 TRX	
G155		Proteo-bacteria	Gamma-proteob.	Alteromona-daceae	*Alteromonas sp.*	Eub338 DY415	Gam42a Atto647N	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Vib2300 TRX	Vib1749 TRX	
G159	PE	Proteo-bacteria	Gamma-proteob.	Alteromona-daceae	*Alteromonas* sp.	Eub338 DY415							Vib1749 TRX	
G156	PS	Proteo-bacteria	Gamma-proteob.	Alteromona-daceae	*Marinobacter*	Eub338 DY415	Gam42a CY5	Gam42a DY647P1						
G158	PLA	Proteo-bacteria	Gamma-proteob.	Alteromona-daceae	*Marinobacter hydrocarbonoclasticus*	Eub338 DY415					Alt811 RRX			

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G161	PP	Proteo-bacteria	Gamma-proteob.	Alteromona-daceae	*Marinobacter hydrocarbonoclasticus*	Eub338 DY415	Gam42a DY505	Alf968 Atto620						
A702	Plas.	Proteo-bacteria	Gamma-proteob.	Halomona-daceae	*Cobetia*	Eub338 DY415	Gam42a CY5	Gam42a DY647P1						
G169	PE	Proteo-bacteria	Gamma-proteob.	Halomona-daceae	*Halomonas* sp.	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Vib2300 TRX	Vib1749 TRX	Gam42a Atto647N
G193	PP	Proteo-bacteria	Gamma-proteob.	Halomona-daceae	*Halomonas zincidurans*	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Vib2300 TRX	Vib1749 TRX	Gam42a Atto647N
G196	PE	Proteo-bacteria	Gamma-proteob.	Halomona-daceae	*Halomonas zincidurans*	Eub338 DY415				Bac1058 RRX				
G162	PP	Proteo-bacteria	Gamma-proteob.	Idiomarina-ceae	*Idiomarina baltica*	Eub338 DY415	Gam42a DY505	Alf968 Atto620						
G168	PS	Proteo-bacteria	Gamma-proteob.	Idiomarina-ceae	*Idiomarina* sp.	Eub338 DY415	Gam42a Atto647N	Alf968 Atto620						
B141		Proteo-bacteria	Gamma-proteob.	Oceanospi-rillaceae	*Amphritea*	Eub338 DY415						Vib2300 TRX	Vib1749 TRX	
A196		Proteo-bacteria	Gamma-proteob.	Oceanospi-rillaceae	*Marinomonas*	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Vib2300 TRX	Vib1749 TRX	
A251		Proteo-bacteria	Gamma-proteob.	Oceanospi-rillaceae	*Marinomonas*	Eub338 DY415							Vib1749 TRX	
A322		Proteo-bacteria	Gamma-proteob.	Oceanospi-rillaceae	*Marinomonas*	Eub338 DY415	Gam42a CY5	Gam42a DY647P1						
A332		Proteo-bacteria	Gamma-proteob.	Oceanospi-rillaceae	*Marinomonas*	Eub338 DY415	Gam42a CY5	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505		Vib1749 TRX	
G147		Proteo-bacteria	Gamma-proteob.	Pseudoalte-romadaceae	*Pseudoalteromonas*	Eub338 DY415					Alt811 DY505	Vib2300 TRX	Vib1749 TRX	
G163	PE	Proteo-bacteria	Gamma-proteob.	Pseudoalte-romadaceae	*Pseudoalteromonas shioyasakiensis*	Eub338 DY415	Gam42a DY505	Alf968 Atto620						
B047		Proteo-bacteria	Gamma-proteob.	Pseudomo-nadaceae		Eub338 DY415	Gam42a DY505	Alf968 Atto620						
G062	PLA	Proteo-bacteria	Gamma-proteob.	Vibriona-ceae	*Enterovibrio* sp.	Eub338 DY415	Gam42a CY5	Gam42a DY647P1						
G079	PLA	Proteo-bacteria	Gamma-proteob.	Vibriona-ceae	uncultured *Vibrio* sp.	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Gam42a Atto647N	Vib1749 TRX	Gam42a CY5
G091	PS	Proteo-bacteria	Gamma-proteob.	Vibriona-ceae	uncultured *Vibrio* sp.	Eub338 DY415	Gam42a DY505	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505	Vib2300 TRX	Vib1749 TRX	
G130		Proteo-bacteria	Gamma-proteob.	Vibriona-ceae	*Vibrio anguillarum*	Eub338 DY415	Gam42a DY505	Alf968 Atto620				Vib2300 TRX	Vib1749 TRX	

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ID	from PMD	Phylum	Class	Family	Genus	Tested probes								
G040	PHA	Proteo-bacteria	Gamma-proteob.	Vibriona-ceae	*Vibrio parahaemolyticus*	Eub338 DY415	Gam42a CY5	Alf968 Atto620	Rho1682 DY490	Bac1058 RRX	Alt811 DY505		Vib1749 TRX	
G082	PLA	Proteo-bacteria	Gamma-proteob.	Vibriona-ceae	*Vibrio* sp.	Eub338 DY415						Vib2300 TRX	Vib1749 TRX	

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Table S3: Quantification of probe fluorescence intensity on target and non-target taxa. The full probe set was applied to pure cultures and imaged (Figure 2) using identical imaging settings for each culture. Unmixed images corresponding to each fluorophore were segmented to identify cells and mean intensity of each fluorophore was measured for each cell. Cell numbers considered for fluorescence intensity calculation is provided under taxon name. Mean intensity (+/- standard deviation) across all cells is reported in arbitrary 16-bit grayscale units ranging from 0 (black) to 65,535 (white). For hybridization of probes to non-targets, the relative intensity is also shown as a percentage of the maximum mean on-target hybridization intensity.

	Eub338 I DY415	Alf968 Atto620	Rho1682 DY490	Gam42a CY5	Alt811 DY505	Vib2300 Texas RedX	Bac1058 Rhodamine RedX
Alphaproteo-bacteria Erythro-bacteraceae 1185 cells	10 677 (± 3192)	2287 (± 688)	43 (± 61) 0.2 %	69 (± 53) 0.8 %	291 (± 344) 3.6 %	120 (± 65) 0.6 %	198 (± 166) 8.8 %
Alphaproteo-bacteria Rhodo-bacteraceae 1493 cells	7424 (± 2403)	5738 (± 2177)	20 867 (± 8420)	103 (± 85) 1.2 %	1196 (± 632) 15 %	82 (± 57) 0.4 %	19 (± 27) 0.8 %
Gammaproteo-bacteria, Halomona-daceae 187 cells	14 540 (± 4574)	2412 (± 872) 42 %	9 (± 14) 0.04 %	5863 (± 1853)	4 (± 15) 0.05 %	237 (± 1977) 1.2 %	2 (± 4) 0.08 %

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	Eub338 I DY415	Alf968 Atto620	Rho1682 DY490	Gam42a CY5	Alt811 DY505	Vib2300 Texas RedX	Bac1058 Rhodamine RedX
Gammaproteo-bacteria, Alteromona-daceae 9552 cells	11 013 (± 3418)	2380 (± 815) 42 %	711 (± 923) 3.4 %	8351 (± 2885)	8199 (± 2369)	19 334 (± 7090) 96 %	18 (± 22) 0.8 %
Gammaproteo-bacteria, Vibrionaceae 7914 cells	13 465 (± 3611)	838 (± 496) 15 %	9 (± 24) 0.05 %	8521 (± 2300)	13 (± 50) 0.2 %	20 033 (± 5644)	25 (± 41) 1.1 %
Bacteroidetes, Flammeo-virgaceae 6756 cells	4892 (± 1218)	1040 (± 461) 18 %	62 (± 51) 0.3 %	202 (± 59) 2.4 %	59 (± 41) 0.7 %	65 (± 40) 0.3 %	2237 (± 571)

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Figure S1: Plots and correlation analyses of microscope counts and amplicon reads after week 1 (correlation coefficient = 0.957) and week 2 (correlation coefficient = 0.990). In both cases there was a significant positive relationship, (n = 6, P < 0.01).