

Specificities and Efficiencies of Primers Targeting *Candidatus Phylum Saccharibacteria* in Activated Sludge

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Supplementary Materials:

(A) Primer TM7314F

Target (5'-3')	G	A	G	A	G	G	A	T	G	A	T	C	A	G	C	C	A	G
A	0	1302	3	1298	26	4	1263	3	1	1294	5	0	1284	3	1	0	1251	1
T	1	0	0	0	1	0	30	1294	0	0	1050	0	1	98	191	0	49	1
G	1300	0	1296	4	1274	1296	9	0	1301	7	0	0	6	1198	1	1	2	1143
C	1	0	3	1	2	1	1	4	0	1	246	1303	12	3	1110	1302	1	158
Other*	1	1	1	0	0	2	0	2	1	1	2	0	0	1	0	0	0	0
Coverage (%)	99	99	99	99	97	99	96	99	99	99	80	100	98	91	85	99	96	87
	Average 95.6%																	

(B) Primer TM7580F

Target (5'-3')	A	Y	T	G	G	G	C	G	T	A	A	A	G	A	G	T	T	G	C
A	1301	1	1	0	2	1	0	1	0	1301	1302	1298	3	1237	0	10	1	5	1
T	0	16	1300	0	1	96	0	1300	1	0	0	0	1	5	1292	1240	7	28	
G	2	0	1	1303	1301	1298	1	1300	1	0	1	1	1300	3	1298	0	1	1285	1
C	0	1286	1	0	0	2	1206	2	1	0	0	0	0	62	0	1	3	6	1273
Other*	0	0	0	0	1	0	1	3	0	4	0	0	0	0	58	0	0	0	0
Coverage (%)	99	98	99	100	99	99	92	99	99	99	99	99	99	94	99	99	95	98	91
	Average 98.0%																		

(C) Primer Sac1031F

Target (5'-3')	A	A	G	A	G	A	A	C	T	G	T	G	C	C	T	T	C	G	G
A	1298	1302	2	194	944	312	993	135	669	0	1	18	48	12	9	1	491	57	101
T	0	0	65	238	22	62	214	142	568	30	1292	0	33	522	1195	1267	409	791	472
G	5	0	1145	643	136	926	82	674	31	1263	5	1229	1	14	53	3	18	409	726
C	0	0	91	228	201	2	12	352	35	3	3	47	1211	749	45	31	330	5	2
Other*	0	1	0	0	0	1	2	0	0	7	2	9	10	6	1	1	55	41	2
Coverage (%)	99	99	87	14	10	23	76	27	43	98	99	94	92	57	91	97	25	31	55
	Average 63.9%																		

(D) Primer 400F

Target (5'-3')	T	A	T	G	A	G	T	G	A	A	G	A	A	T	A	T	G	A	C
A	0	1148	29	631	739	34	45	0	1300	796	93	1300	865	35	1180	11	1	1299	1
T	1301	15	1264	169	504	21	1005	0	1	51	3	2	407	1266	113	1276	0	0	91
G	0	74	3	324	47	1217	70	1301	2	227	1206	0	5	1	4	2	1301	3	0
C	2	66	7	179	12	31	182	1	0	228	0	0	24	1	5	14	0	0	1211
Other*	0	0	0	0	1	0	1	0	1	1	1	2	0	1	0	1	1	0	
Coverage (%)	99	88	97	24	56	93	77	99	99	61	92	99	66	97	90	97	99	99	92
	Average 85.5%																		

(E) Primer TM7-910R

Target (5'-3')	C	A	T	A	A	A	G	G	A	A	T	T	G	A	C	G	G	A	C	
A	0	1156	0	1302	1302	1299	0	0	1301	1300	0	6	0	1302	0	3	2	1	1286	2
T	0	143	1164	0	0	0	0	0	0	1300	1293	0	1	0	1	1	0	3	24	
G	0	1	0	1	3	1303	1303	1	3	0	1	1303	0	0	1299	1299	1301	1302	4	
C	1303	2	139	0	0	0	0	0	0	0	0	1	3	0	0	1303	0	1	0	
Other*	0	1	0	0	0	1	0	0	1	0	2	0	0	0	0	0	0	1	1250	
Coverage (%)	100	88	89	99	99	99	99	100	99	99	99	99	100	99	99	99	99	98	95	
	Average 98.0%																			

(F) Primer TM7-1177R

Target (5'-3')	G	G	A	A	G	G	A	G	G	G	A	T	G	A	T	G	C	A	G	G	T	C	
A	2	2	1303	1290	1	2	1101	4	5	0	0	1294	5	1	1303	16	2	2	0	1274	6	1	0
T	1	0	0	2	0	1	49	0	1	0	0	5	1256	1	0	1106	4	1283	5	24	1	0	1299
G	1299	1301	0	6	1301	1295	146	1298	1297	1302	1302	3	3	1301	0	30	1297	0	1	1294	1301	0	
C	0	0	0	2	1	2	3	0	0	0	0	0	0	0	0	150	0	14	1297	4	2	0	
Other*	1	0	0	3	0	3	4	1	0	1	1	0	0	0	0	1	0	4	0	0	1	0	
Coverage (%)	99	99	100	99	99	99	84	99	99	99	99	96	99	100	84	99	98	99	97	99	99	98	
	Average 97.6%																						

(G) Primer Sac1218R

Target (5'-3')	G	G	T	C	A	G	T	A	T	T	T	T	C	C	C	T	T	A	C	G	C
A	6	1	0	0	1201	2	0	1300	0	0	394	6	0	1	1	0	1298	1	461	3	
T	1	0	1299	0	32	53	1235	1	1302	1214	714	4	176	4	1297	1299	1	60	0	849	
G	1294	1301	2	0	29	1248	0	2	0	68	194	46	11	1	1	0	2	0	839	1	
C	2	0	2	1303	41	0	67	0	1	20	1	1247	1115	1296	3	4	2	1242	3	449	
Other*	0	1	0	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	1	0	
Coverage (%)	99	99	99	100	92	95	94	99	99	93	54	95	85	99	99	99	99	95	64	34	
	Average 89.6%																				

(H) Primer 1110R

Target (5'-3')	G	T	A	T	T	T	T	C	T	A	C	T	T	G	G	A	C	T	G

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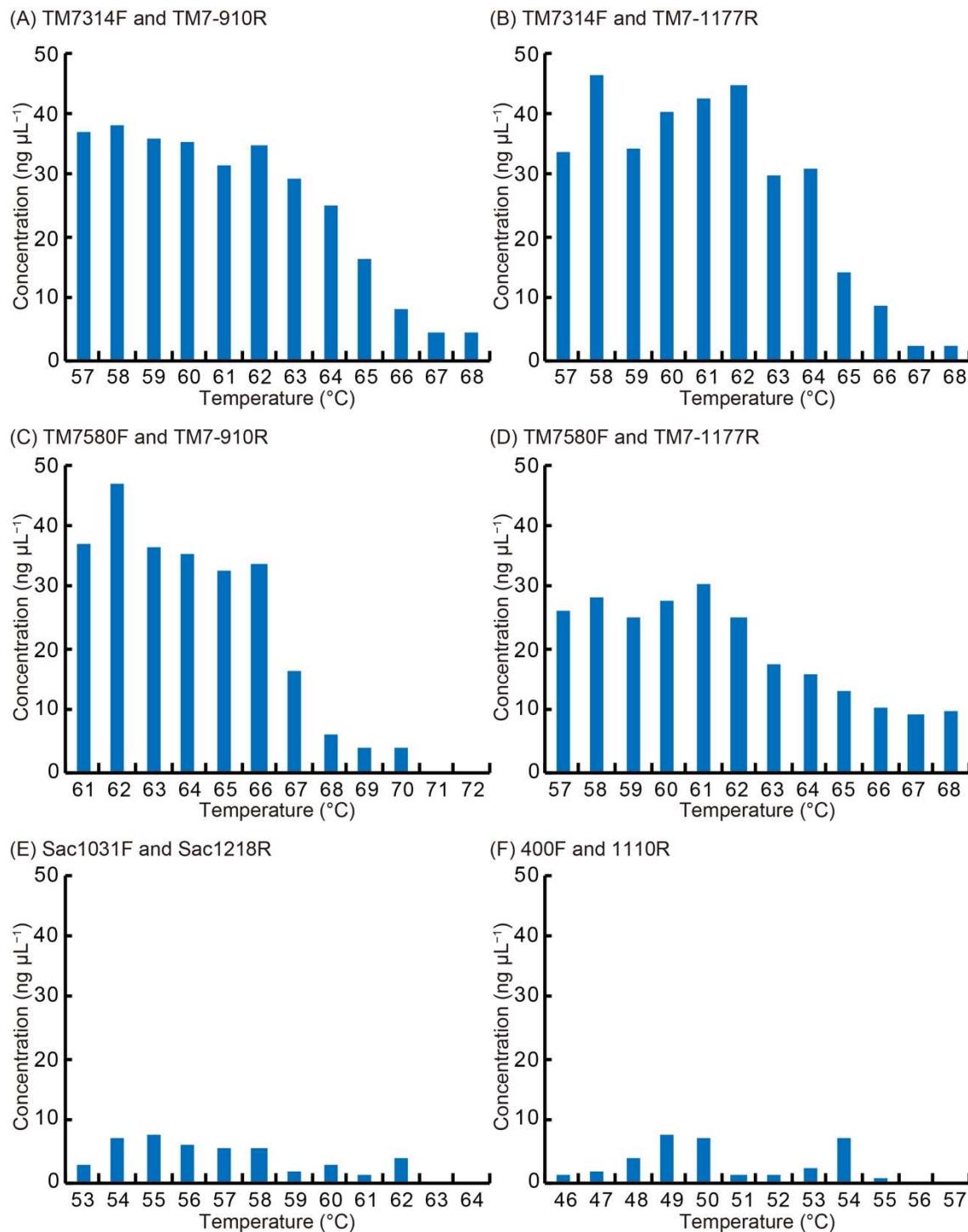


Figure S2. Concentration of PCR product using the primer set with TM7314F and TM7-910R (A), TM7314F and TM7-1177R (B), TM7580F and TM7-910R (C), TM7580F and TM7-1177R (D), Sac1031F and Sac1218R (E), and 400F and 1110R (F).

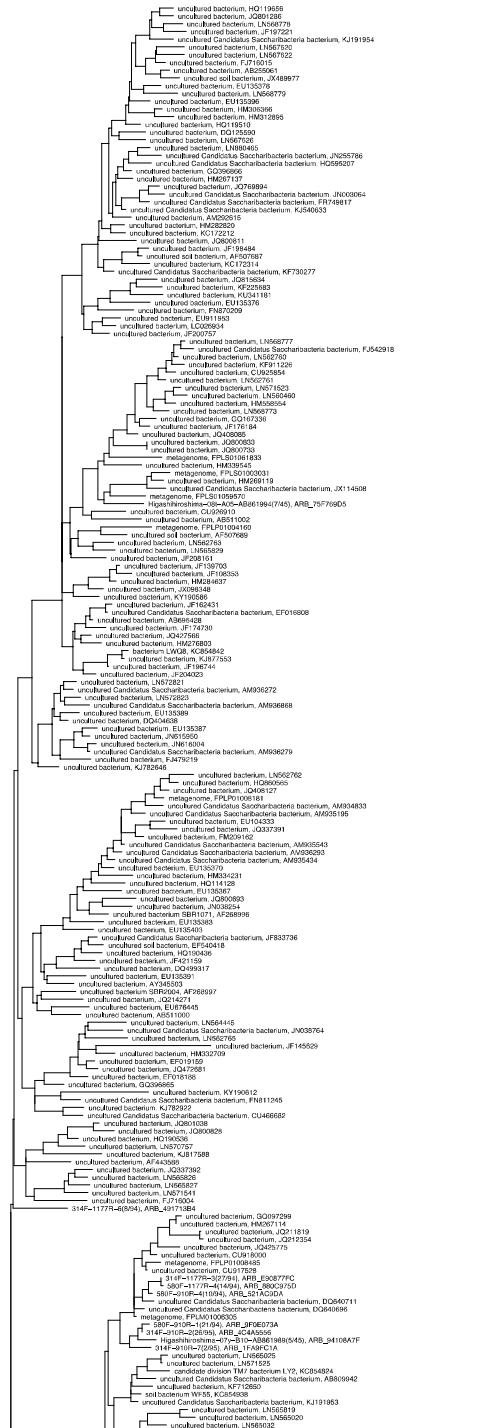


Figure S3. Phylogenetic tree of 1,303 Saccharibacteria sequences and related operational taxonomic units (OTUs) obtained from activated sludge. *Thermotoga* sequences were used as an outgroup.

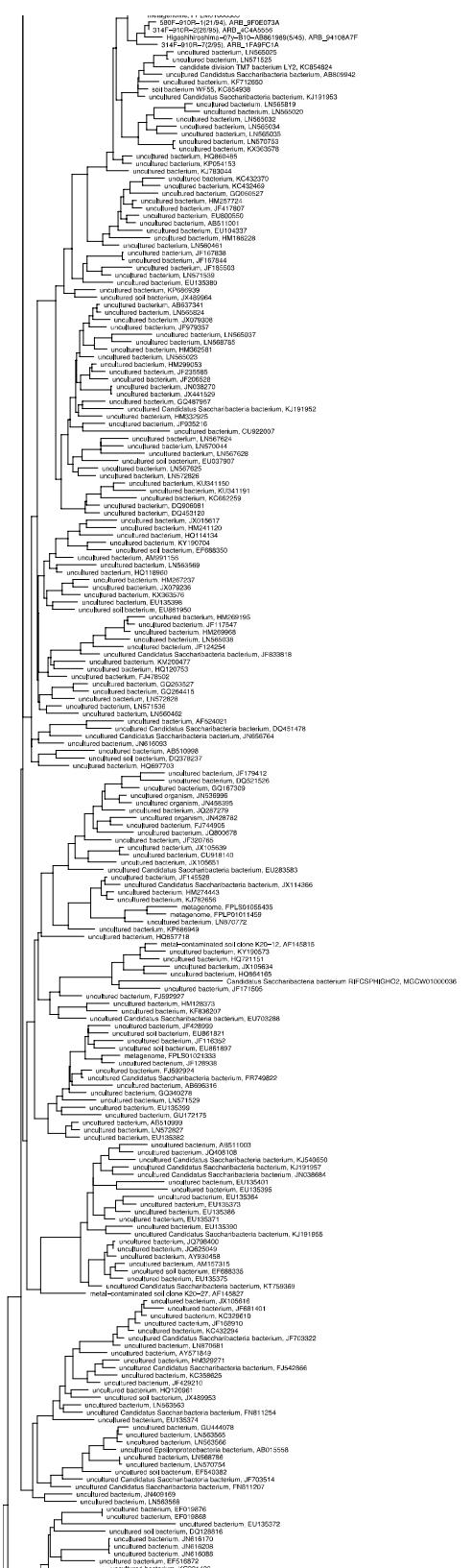


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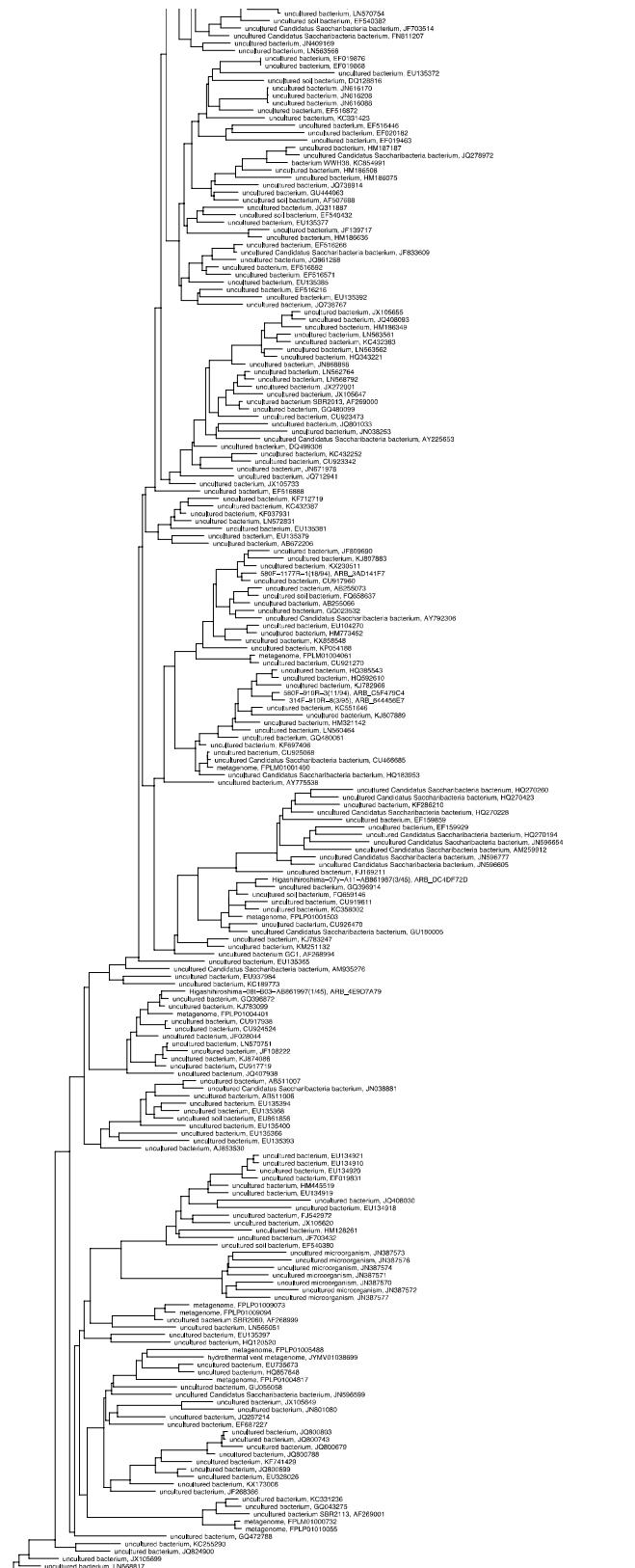


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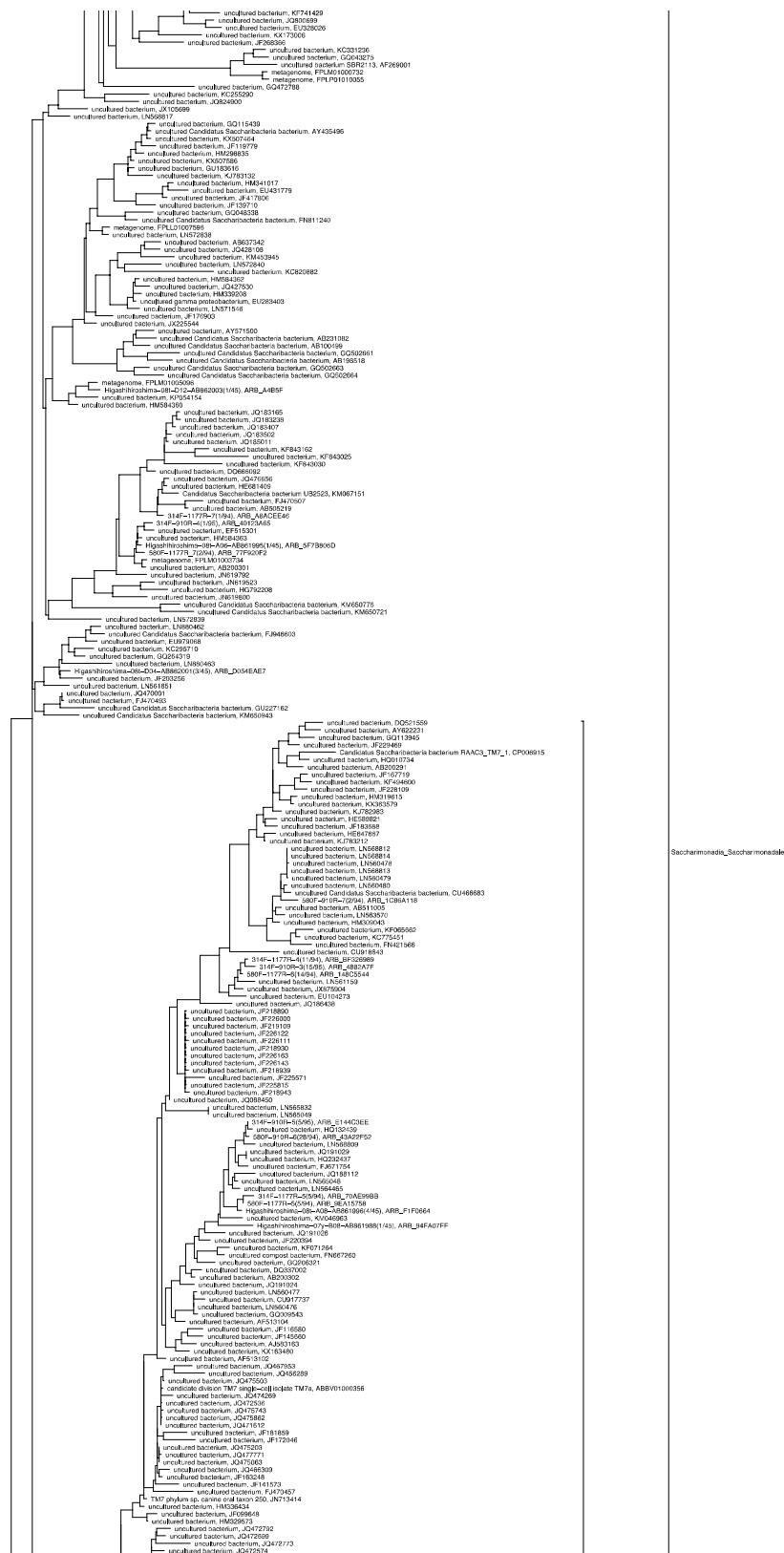


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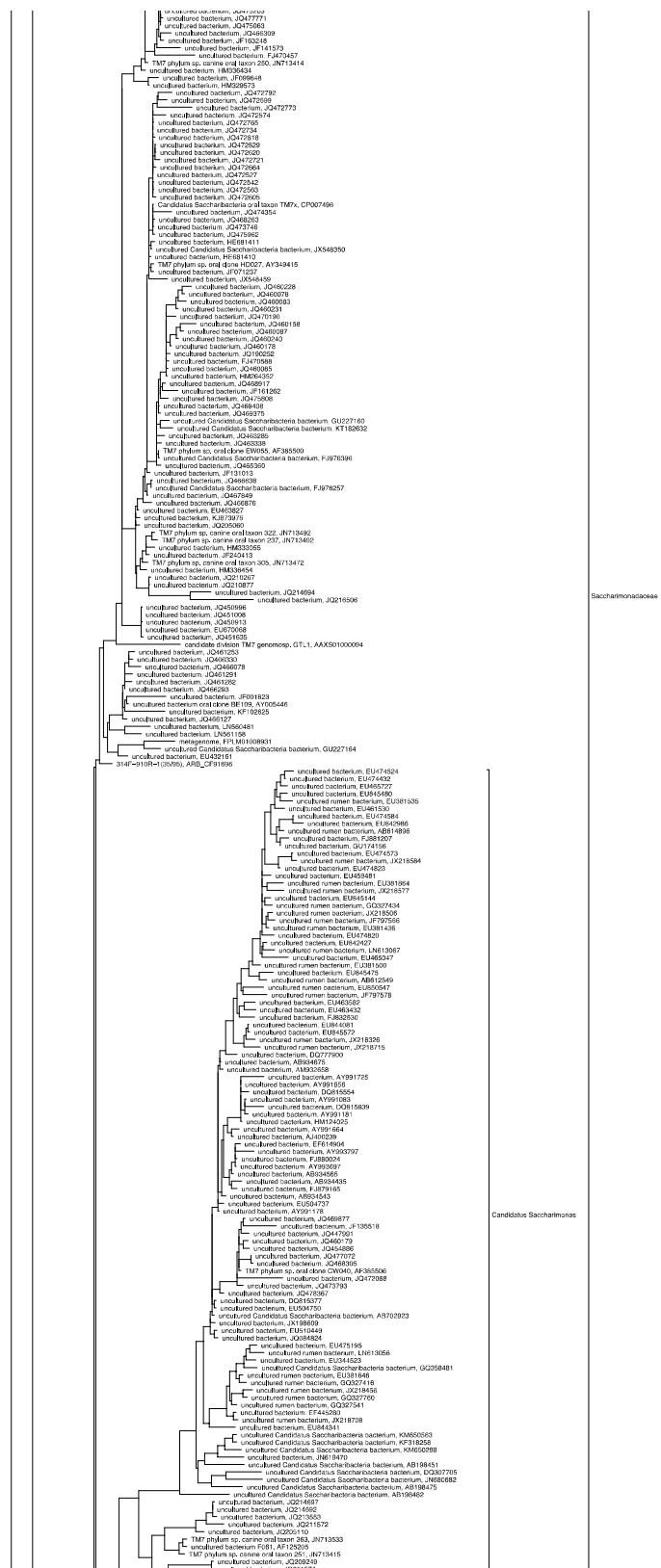


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Figure S3. Continued.

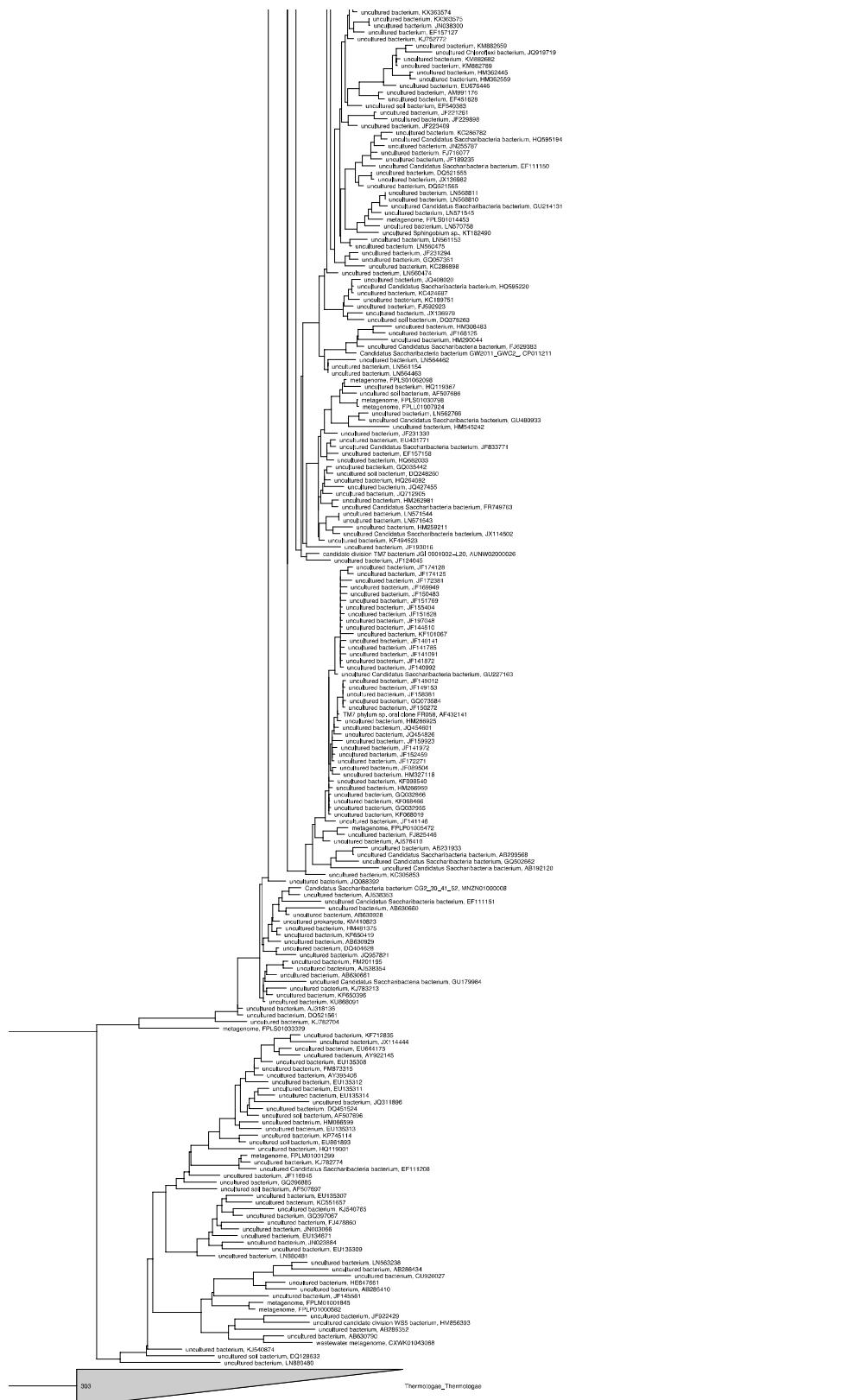


Figure S3. Continued.

Table S1. Phylogenetic relatives of the OTUs analyzed in this study.

OTU	Closest relatives	Accession No.	Identity (%)
TM7314F-TM7-910R_1 (35/95)	Uncultured bacterium	EU432161	96.7
TM7314F-TM7-910R_2 (26/95)	Metagenome	FPLM01006305	96.6
TM7314F-TM7-910R_3 (15/95)	Uncultured bacterium	JX875904	97.4
TM7314F-TM7-910R_4 (1/95)	Uncultured bacterium	EF515301	97.7
TM7314F-TM7-910R_5 (5/95)	Uncultured bacterium	HQ132439	99.8
TM7314F-TM7-910R_6 (8/95)	<i>Candidatus Saccharimonas aalborgensis</i>	CP005957	99.8
TM7314F-TM7-910R_7 (2/95)	metagenome	FPLM01006305	96.6
TM7314F-TM7-910R_8 (3/95)	Uncultured bacterium	HQ385543	95.7
TM7314F-TM7-1177R_1 (15/94)	<i>Candidatus Saccharimonas aalborgensis</i>	CP005957	99.7
TM7314F-TM7-1177R_2 (27/94)	uncultured soil bacterium	AF525834	95.0
TM7314F-TM7-1177R_3 (27/94)	Uncultured <i>Candidatus Saccharibacteria</i> bacterium	DQ640711	98.6
TM7314F-TM7-1177R_4 (11/94)	Uncultured bacterium	JX875904	98.5
TM7314F-TM7-1177R_5 (5/94)	Uncultured bacterium	KM046963	94.3
TM7314F-TM7-1177R_6 (8/94)	Uncultured bacterium	LN571536	92.6
TM7314F-TM7-1177R_7 (1/94)	Uncultured bacterium	JQ476656	99.3
TM7580F-TM7-910R_1 (21/94)	Metagenome	FPLM01006305	94.3
TM7580F-TM7-910R_2 (12/94)	<i>Candidatus Saccharimonas aalborgensis</i>	CP005957	100.0
TM7580F-TM7-910R_3 (11/94)	Uncultured bacterium	HQ385543	95.4
TM7580F-TM7-910R_4 (10/94)	Uncultured <i>Candidatus Saccharibacteria</i> bacterium	DQ640711	98.9
TM7580F-TM7-910R_5 (10/94)	Uncultured soil bacterium	AF525834	96.5
TM7580F-TM7-910R_6 (28/94)	Uncultured bacterium	HQ132439	99.5
TM7580F-TM7-910R_7 (2/94)	Uncultured bacterium	LN568812	97.0
TM7580F-TM7-1177R_1 (18/94)	Uncultured bacterium	CU917960	97.4
TM7580F-TM7-1177R_2 (24/94)	<i>Candidatus Saccharimonas aalborgensis</i>	CP005957	99.8
TM7580F-TM7-1177R_3 (17/94)	Uncultured soil bacterium	AF525834	93.6
TM7580F-TM7-1177R_4 (14/94)	Uncultured <i>Candidatus Saccharibacteria</i> bacterium	DQ640711	98.6
TM7580F-TM7-1177R_5 (5/94)	Uncultured bacterium	KM046963	93.9
TM7580F-TM7-1177R_6 (14/94)	Uncultured bacterium	JX875904	98.4
TM7580F-TM7-1177R_7 (2/94)	Uncultured bacterium	HM584363	98.7

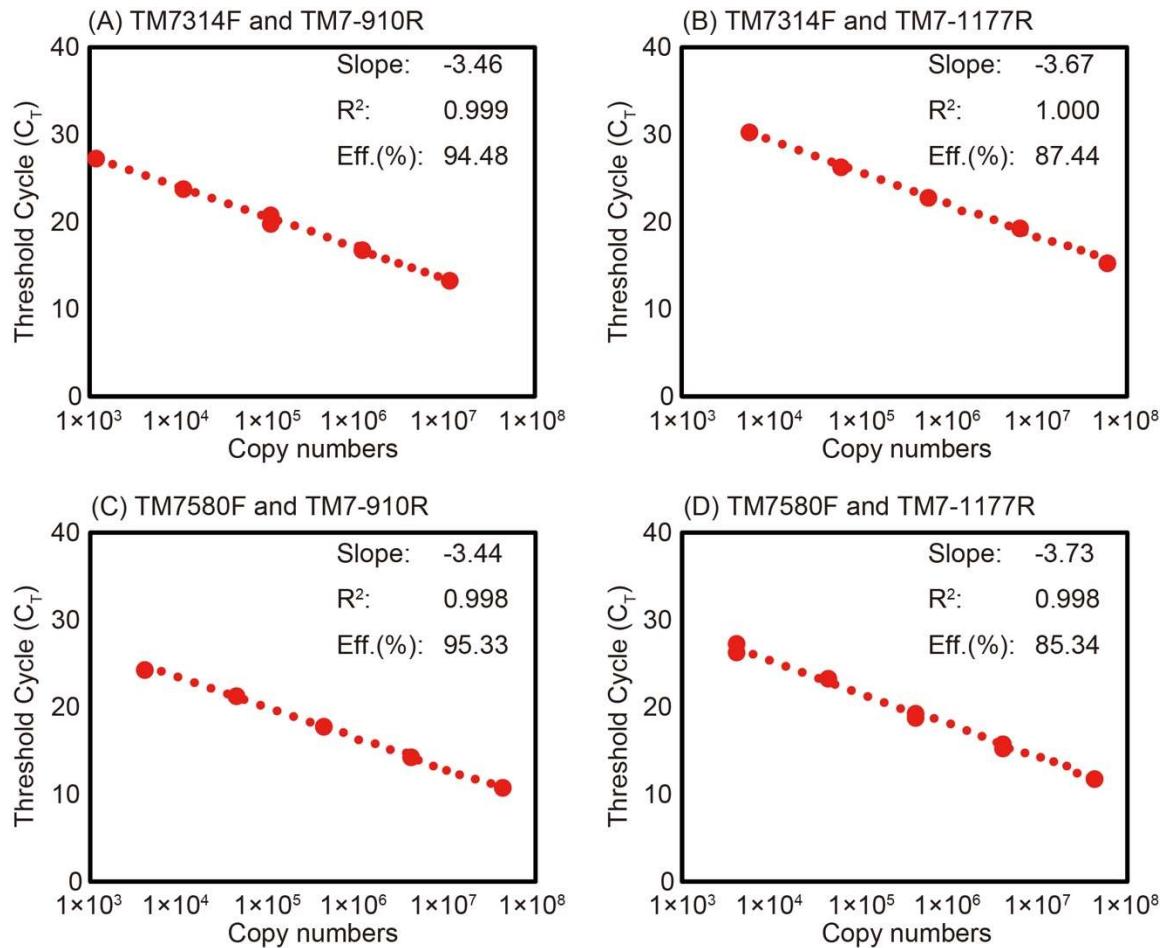


Figure S4. Standard curves of Saccharibacteria qPCR for the measurement of copy number of plasmids using 10-fold serial dilutions of plasmid DNA carrying Saccharibacteria 16S rRNA genes and the four primer sets: TM7314F and TM7-910R (A); TM7314F and TM7-1177R (B); TM7580F and TM7-910R (C); and TM7580F and TM7-1177R (D). The slope, coefficient of determination (R^2), and amplification efficiency were also shown in the figures.

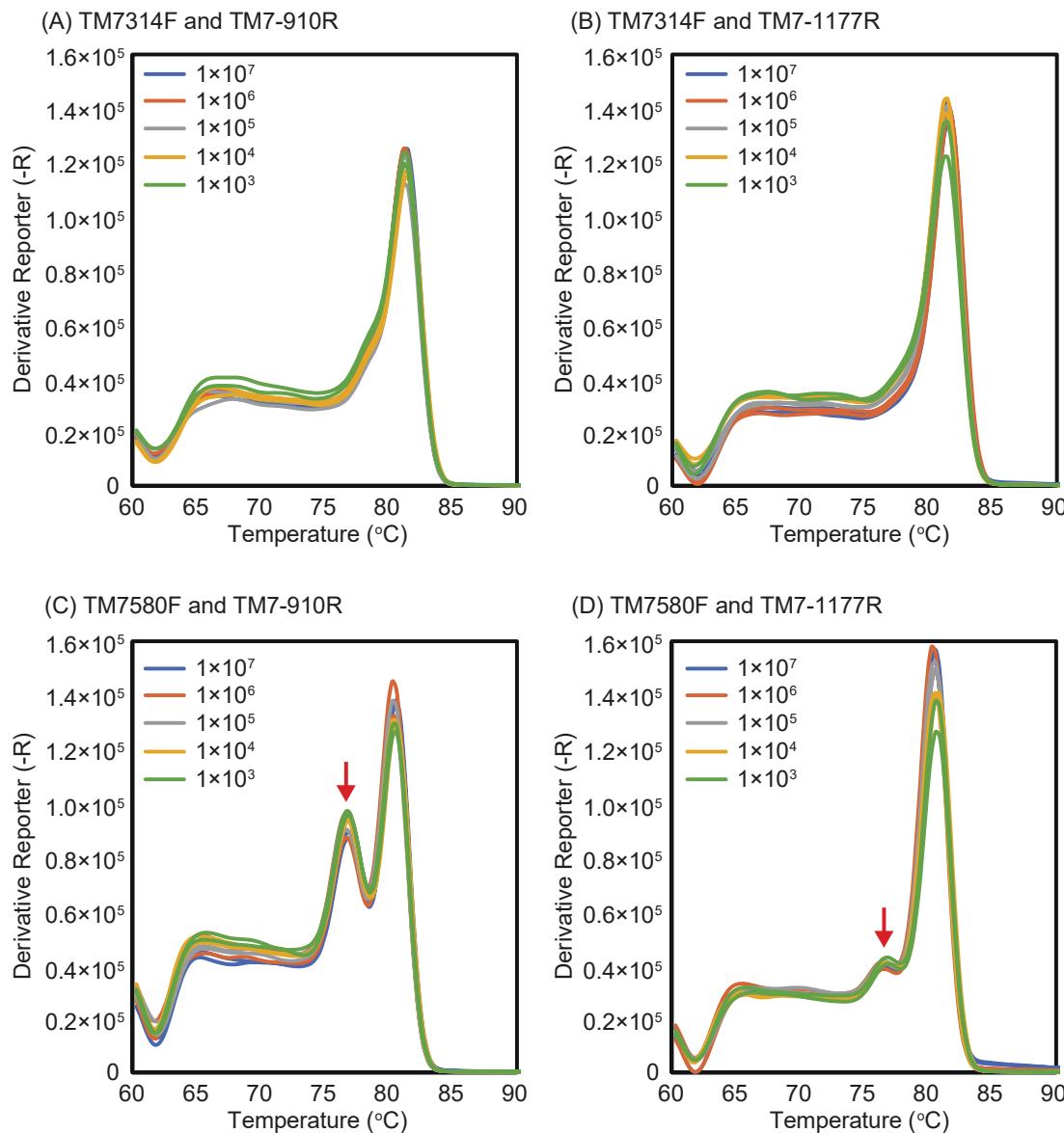


Figure S5. Melting curves of the four standard curves using 10-fold serial dilutions of plasmid DNA carrying *Saccharibacteria* 16S rRNA genes and the four primer sets: TM7314F and TM7-910R (**A**); TM7314F and TM7-1177R (**B**); TM7580F and TM7-910R (**C**); and TM7580F and TM7-1177R (**D**). Red arrows indicate a minor peak, suggesting the presence of non-specific amplification products. In addition to the main peak, another peak at around 77 °C was observed when TM7580F was used (red arrows in (**C**) and (**D**)), suggesting the presence of non-specific amplification products, specifically short products, which were not expected from these primer sets.