Table S1: A list of the tetracycline resistance genes that were included in the three hidden Markov models.

Enzymatic genes		Ribosomal protection genes		Efflux genes	
Protein name	Accession no.	Protein name	Accession no.	Protein name	Accession no.
Tet(X)	AAA27471.1	Otr(A)	CAA37477.1	Tet(D)	CAA46706
Tet(X3)	BAC77725.1	Tet(Q)	CAA79727.1	Tet(B)	AAB59094
Tet(47)	AKQ05891.1	Tet(S)	AAA25293.1	Tet(H)	AAC43250
Tet(48)	AKQ05892.1	TetB(P)	AAA20117.1	Tet(Y)	AAC72341
Tet(49)	AKQ05893.1	Tet(M)	AAA73978.1	Tet(30)	AAD09860
Tet(50)	AKQ05894.1	Tet(T)	AAF01499.1	Tet(G)	AAC98496
Tet(51)	AKQ05895.1	Tet(O)	AAA23033.2	Tet(J)	AAD12753
Tet(52)	AKQ05896.1	Tet(36)	CAD55718.1	Tet(Z)	AAD25063
Tet(53)	AKQ05897.1	Tet(W)	CAA10975.1	Tet(31)	CAC80727
Tet(54)	AKQ05898.1	Tet(32)	ABG36114.2	Tet(33)	CAD12227
Tet(55)	AKQ05899.1	Tet(44)	CBH51823.1	Tet(A)	AAN06707
				Tet(39)	AAW66497
				Tet(41)	AAP93922
				Tet(42)	ACD35503
				Tet(57)	AJO67548
				Tet(59)	AMP42440
				Tet(E)	ABO92308
				Tet(C)	AAK97755

Table S2: Estimated sensitivity and specificity for the three created hidden Markov models.

		Sensitivity		Specificity	
Model	Reference genes	Full-length	Reads (100 nt)	Full-length	Reads (100 nt)
Enzyme	11	1	0.96	1	0.93
RPG	11	1	0.78	1	0.90
Efflux	18	0.94	0.89	1	0.97

Table S4: Distribution of identified previously known tetracycline resistance genes (>80% to any previously known tetracycline resistance gene) in the metagenomic datasets.

	Human Gut	HMP	Pig Gut	WWTP	Pune River	Polluted River
Ribosomal prot.						
Tet(33)			x	x		
Tet(39)				x	x	x
Tet(A)	x	x	x	x		x
Tet(B)	x	x	x		x	
Tet(G)			x		x	
Tet(H)			x			
Efflux						
Tet(32)	x	x	x		x	
Tet(36)	x				x	x
Tet(44)	x	x		x	x	
TetB(P)	x	x	x	x	x	
Tet(W)	x			x	x	
Tet(Q)	x				x	
Tet(T)			x		x	
Tet(S)			x	x		
Tet(O)	x				x	
Tet(M)	x					
Enzymatic						
Tet(X)	x	x		x	x	x

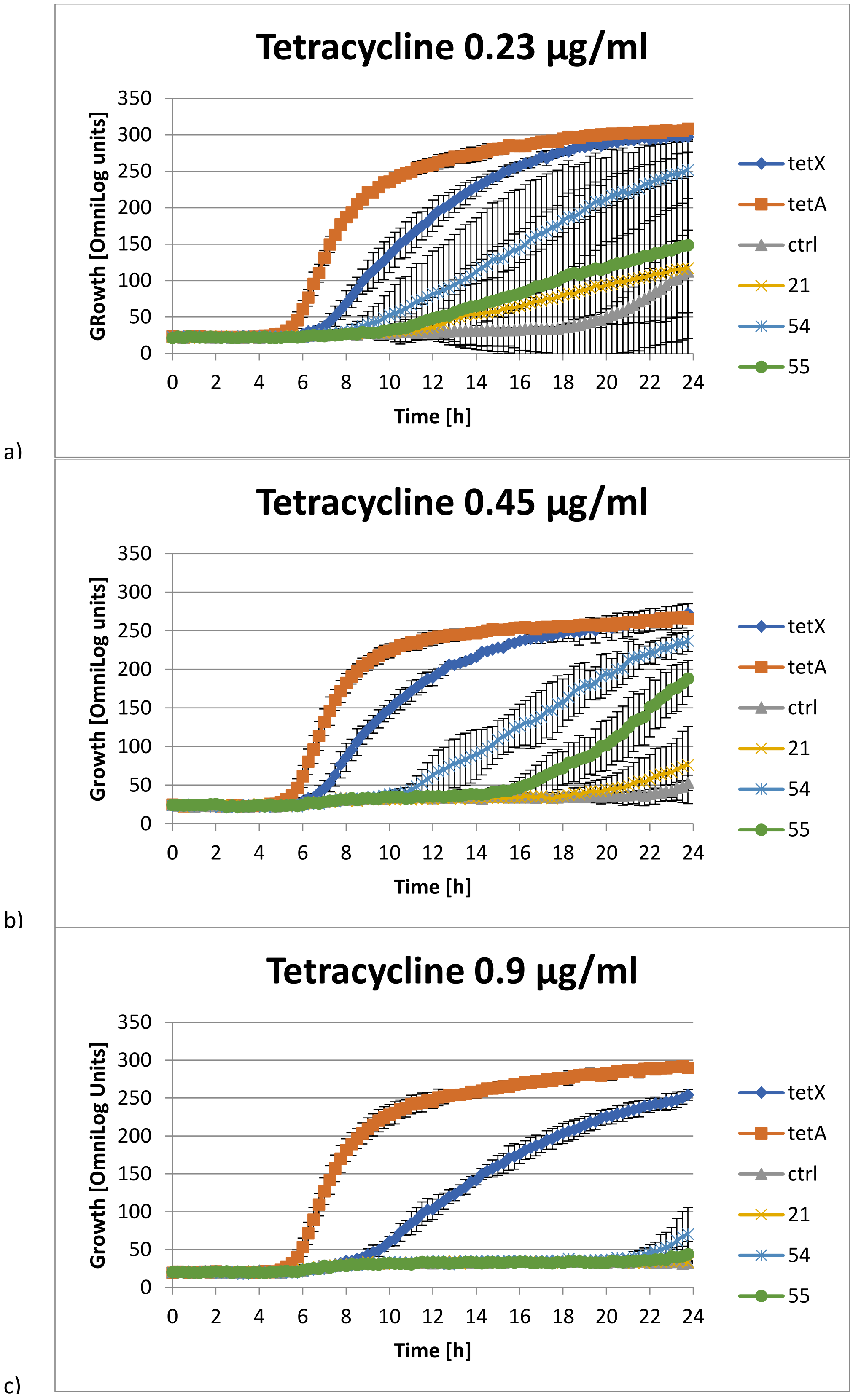
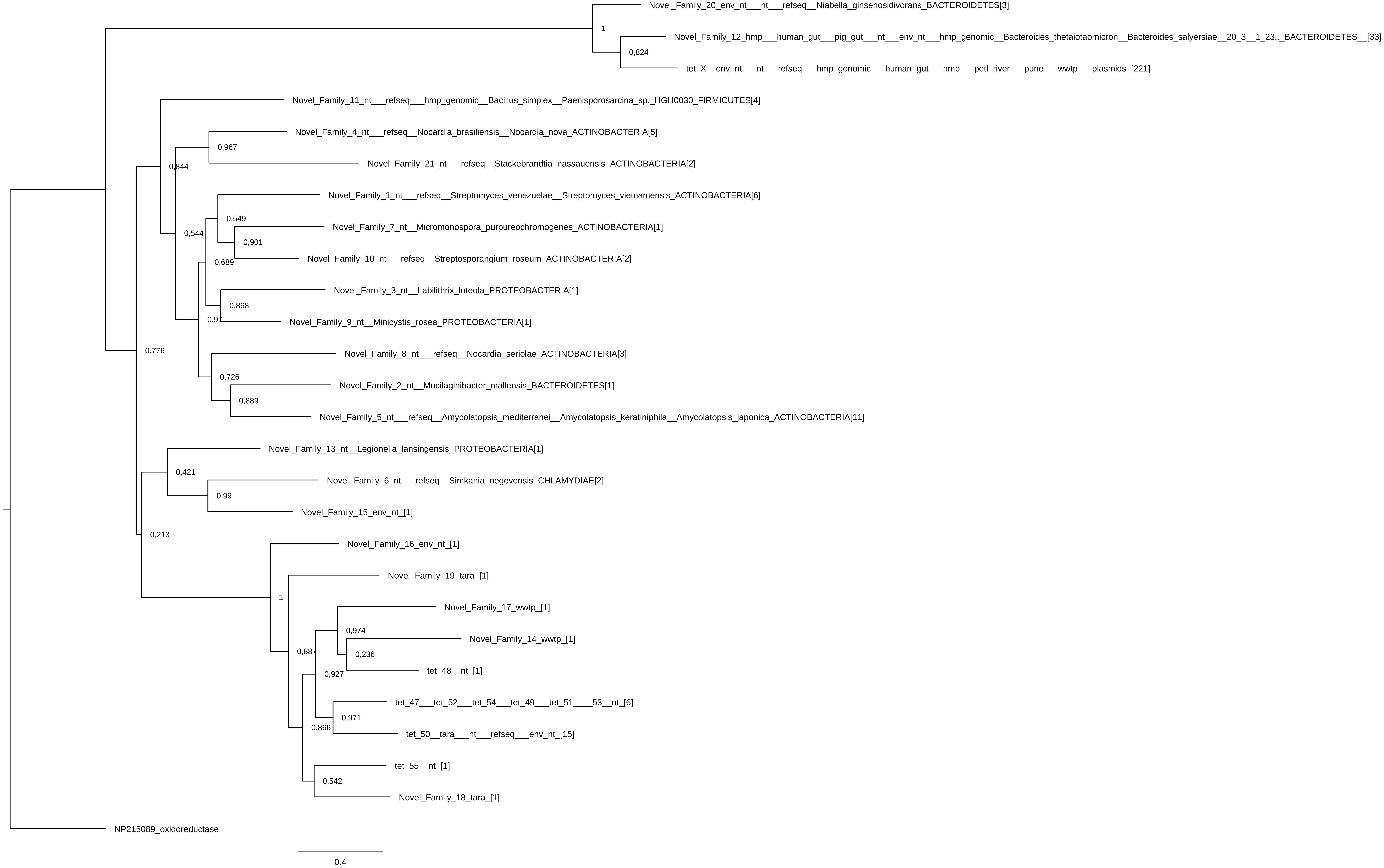


Figure S1: Growth behavior of three constructs (efflux pump G241 in NF21, ribosomal protection G231 in NF54 and ribosomal protection G242 in NF55) compared to Tet(X), Tet(A) and a negative control at three different tetracycline concentrations.

Figure S2: Phylogenetic tree of the enzymatic degradation genes with bootstrap values.

Figure S3: Phylogenetic tree of the ribosomal protection genes with bootstrap values.

Figure S4: Phylogenetic tree of the efflux pump genes with bootstrap values.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Novel_Family_7_nt__Micromonospora_purpureochromogenes_ACTINOBACTERIA[1]
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0,901
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Novel_Family_3_nt__Labilithrix_luteola_PROTEOBACTERIA[1]
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0,97
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Novel_Family_17_nt___refseq__Paenibacillus_mucilaginosus_FIRMICUTES[4]
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Novel_Family_61_env_nt_[2]
Novel_Family_43_env_nt_[1]
Novel_Family_66_env_nt_[1]
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Novel_Family_101_wwtp_[1]
Novel_Family_78_env_nt_[1]
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Novel_Family_33_wwtp___pune_[3]
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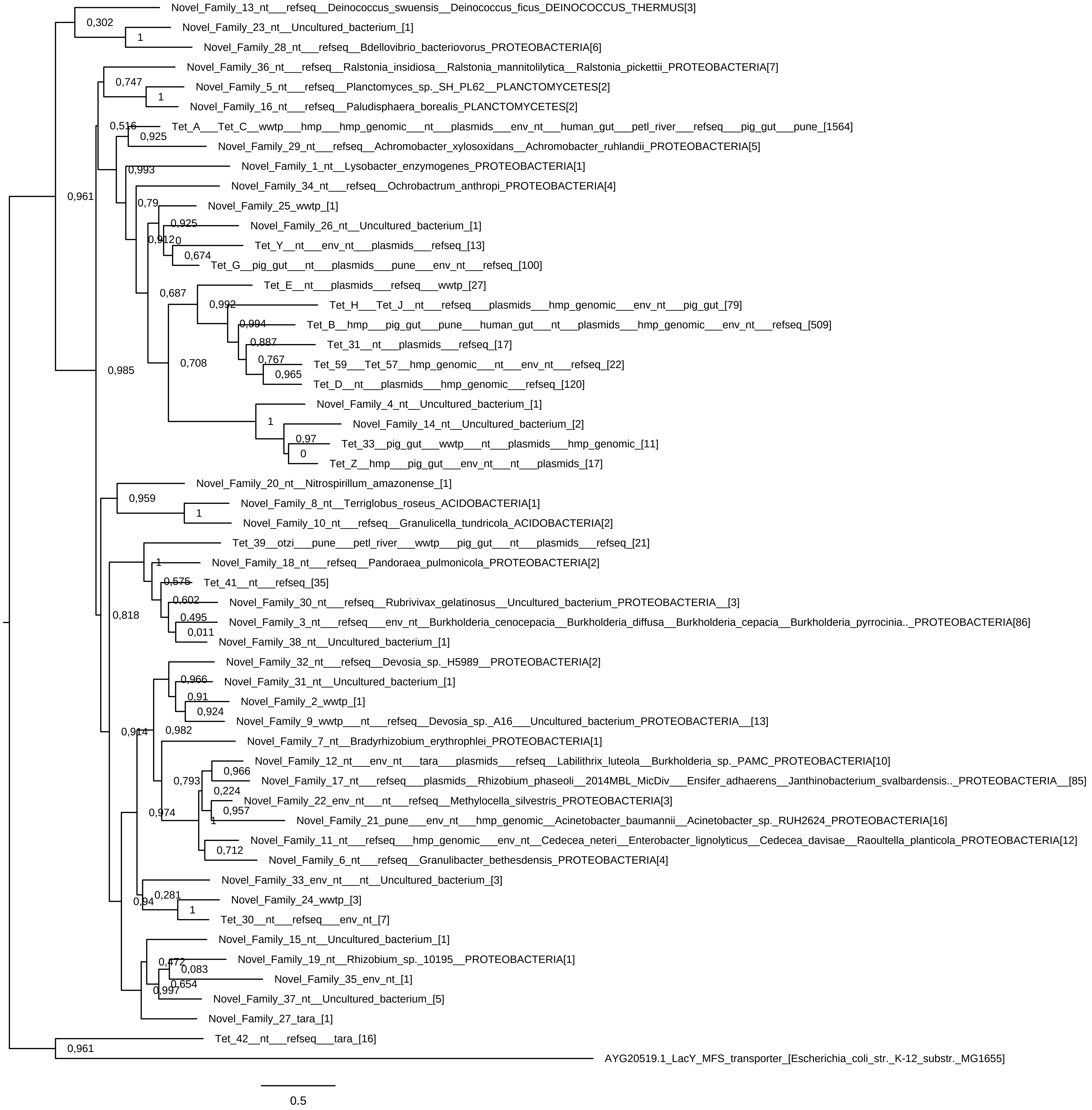
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0,747
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0,79
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0,925
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0,912
0
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0,674
Tet_G__pig_gut___nt___plasmids___pune___env_nt___refseq_[100]
Tet_E__nt___plasmids___refseq___wwtp_[27]
0,687
0,992
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0,957
1
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0,712
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0,94
Novel_Family_24_wwtp_[3]
1
Tet_30__nt___refseq___env_nt_[7]
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0,083
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0,654
0,997
Novel_Family_37_nt__Uncultured_bacterium_[5]
Novel_Family_27_tara_[1]
Tet_42__nt___refseq___tara_[16]
0,961
AYG20519.1_LacY_MFS_transporter_[Escherichia_coli_str._K-12_substr._MG1655]
0.5