Supplementary information for

“TurboFold II: RNA Secondary Structure Prediction and Structural Alignment Informed by Multiple Homologs”

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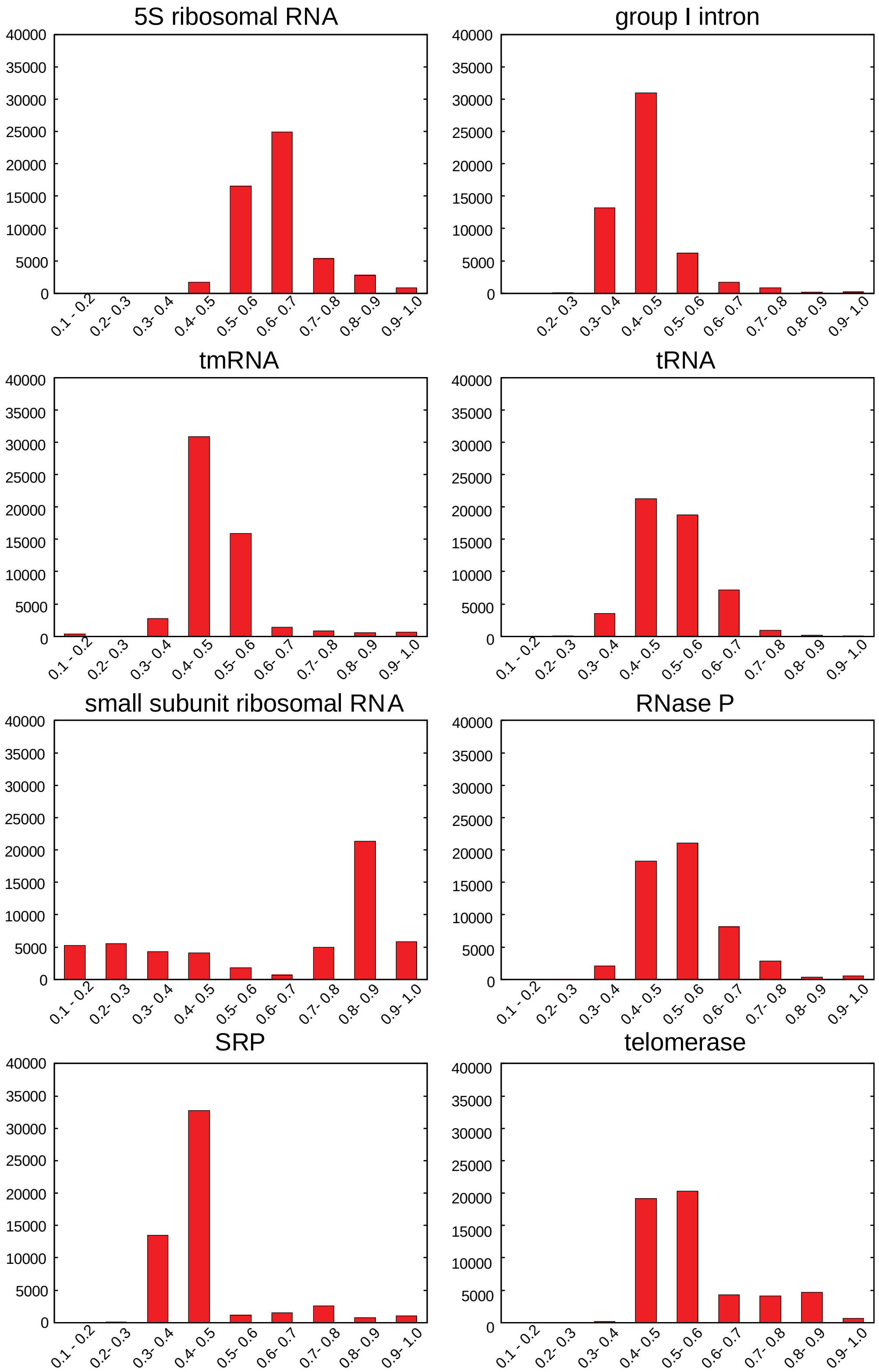
Details of pairwise sequence identity (Section 1), database used in parameter optimization and testing (Section 2), parameter optimization methods (Section 3), sequences being used in parameter optimization and benchmarking (Section 4), tables of values about method performance on alignment and structure predictions (Section 5), benchmark of TurboFold II on BRAliBase 2.0 (Section 6) and accepted database structures and alignments for the example in the Discussion (*Nocardioides albus*, *Propioniferax innocua*, and *Salt Marsh A26(12)*) (section 7) are provided.

Section 1. Pairwise sequence identity:

The pairwise sequence identity between two sequences is computed as the fraction of nucleotide positions for which the nucleotides are aligned and identical over the sum of aligned and inserted positions between the two sequences for the maximum likelihood alignment between the sequences. The sequence identity is computed with a pairwise alignment HMM (1,2). In Figure S1, histograms of pairwise sequence identity are provided for the datasets used in the benchmarks for each individual family: the 5S small subunit rRNA, group I intron RNA, tmRNA, tRNA, small subunit rRNA, RNase P RNA, SRP RNA, and telomerase RNA.


5S ribosomal RNA

group I intron

tmRNA

tRNA

small subunit ribosomal RNA

RNase P

SRP

telomerase

Figure S1. **Histograms for pairwise sequence identity for the datasets used in the benchmarks by each individual family: 5S rRNA, group I intron RNA, tmRNA, tRNA, small subunit rRNA, RNase P RNA, SRP RNA, and telomerase RNA.**

Section 2. Database: RNAStralign

For parameter optimization and benchmarking, an RNA alignment and structure database, named RNAStralign, was aggregated from available online databases of RNA structure and alignment. Structures for each family in RNAStralign are categorized into homologous families based on the classifications in the original databases. If available, further categorization into subfamilies was also included in RNAStralign. Only sequences with known alignments and secondary structures were included. The families included are:

1. Small subunit ribosomal RNA (16S rRNA) family

Small subunit rRNA structures and alignments were downloaded from the Comparative RNA Web Site (3).

2. 5S ribosomal RNA (5S rRNA) family

5S rRNA structures and alignments were downloaded from the 5S Ribosomal RNA Database (4).

3. Group I intron family

Group I intron structures and alignments were downloaded from the Group I Intron Sequence and Structure Database (GISSD) (5). Structures with P1 and P2 domain competition are represented by two structure files which either have P1 or P10 domain and with "_p1" or "_p10" added to the end of their file names.

4. RNase P family

RNase P structures and alignments were downloaded from the RNase P Database (6). Only 7 subfamilies with at least two full length structures (Bacterial Type A RNA, Bacterial Type B RNA, Archaeal RNA, Nuclear RNA, Plastid RNA, Vertibrate Nuclear RNA and Yeast Nuclear RNA) are kept in our database.

5. Signal recognition particle RNA (SRP RNA) family

SRP RNA structures and alignments were downloaded from the Signal Recognition Particle Database (SRPDB) (7).

6. Telomerase RNA family

Telomerase RNA structures and alignment were downloaded from the Rfam database (8) RF00024 family.

7. Transfer-messenger RNA family

Transfer-messenger RNA structures and alignment were downloaded from tmRNA Database (9).

8. Transfer RNA (tRNA) family

tRNA structures and alignments were downloaded from tRNAdb 2009 (10). Because the structures in this database do not include variable loops, covariance models of RNA secondary structure (COVE) (http://eddylab.org/software.html) were used to annotate the variable loop base pairs.

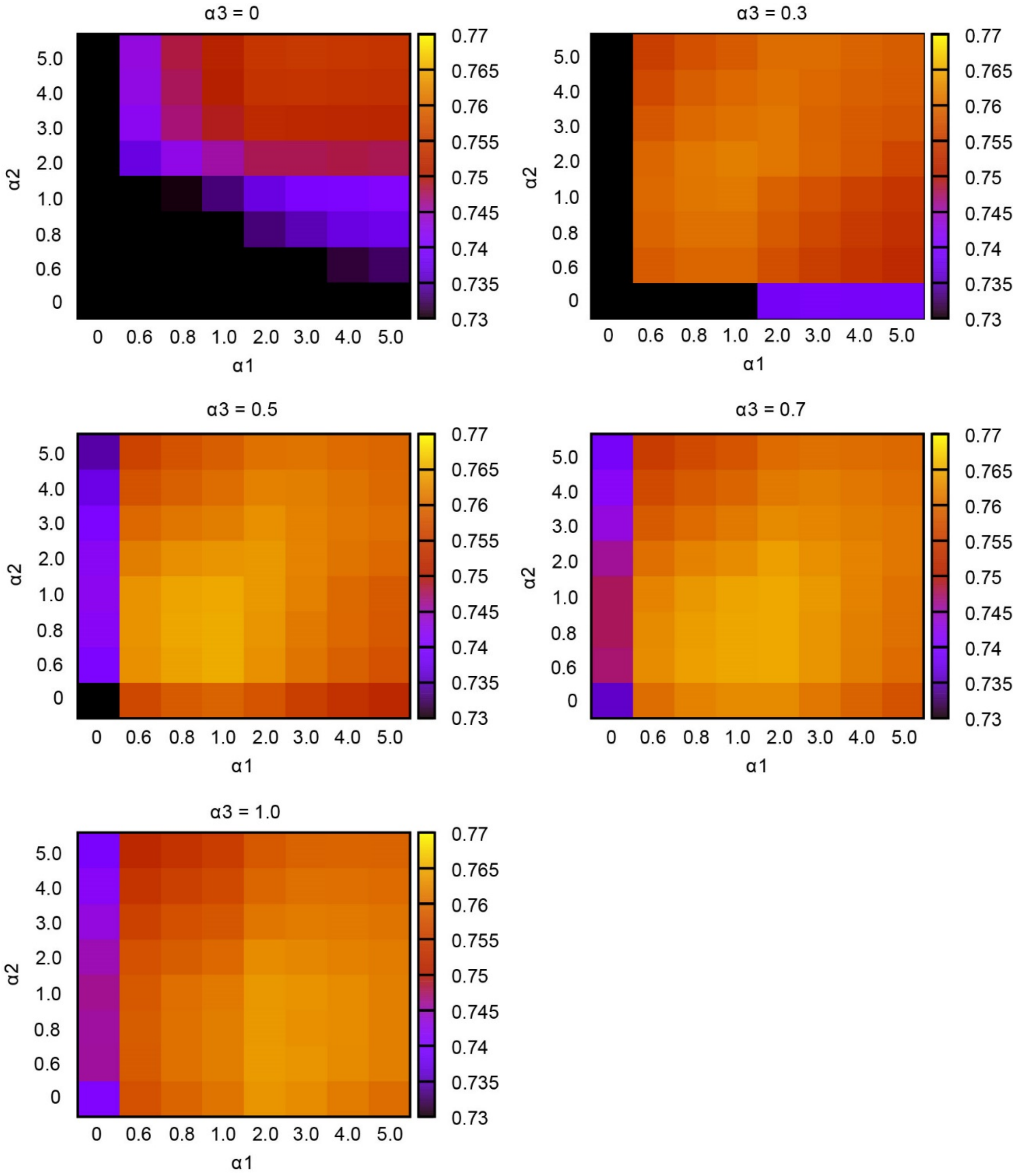
Section 3. Parameter optimization

The match scores for pairwise nucleotides *i* and *k* in two sequences are represented as

$$\rho(i,k) = \alpha_1\left(\sqrt{P_{<}^{m}(i)P_{<}^{n}(k)} + \sqrt{P_{>}^{m}(i)P_{>}^{n}(k)}\right) + \alpha_2\left(\sqrt{P_{\circ}^{m}(i)P_{\circ}^{n}(k)}\right) + \alpha_3$$

To train the three parameters in the match score scheme (α_1, α_2 and α_3), 40 groups of input sequences formed by 3, 5 and 7 homologous sequences, were randomly chosen from the 5S ribosome RNA (Eubacteria subfamily), group I intron (IC1 subfamily), tmRNA, and tRNA from the database RNAStralign.

A grid search was performed to find optimal parameter values. The search range for α_1 and α_2 were both by values of 0, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0, and 5.0. α_3 was searched by values of 0, 0.3, 0.5, 0.7, and 1.0. Figure S2 shows the weighted accuracy of alignment and structure predictions by different parameters. The weighted accuracy is the product of sensitivity and positive predicted value (PPV) of averaged pairwise alignments predictions and averaged structure predictions by the number of input sequences. The sensitivity and PPV calculation are described in the Methods section of the paper. The highest accuracy is achieved by α_1 = 1.0, α_2 = 0.8 and α_3 = 0.5, which were used as the default for the TurboFold II.



α3 = 0

α3 = 0.3

α3 = 0.5

α3 = 0.7

α3 = 1.0

Figure S2. **Grid search plots for parameters α1, α2 and α3 in match score function.** The heat map keys on the right side show that the grid cells with higher values are represented in lighter color.

Section 4. Sequences used in parameters optimization and benchmarking

Sequences were separated in 3, 5, 7, 10, and 20 input sequences for each family (parameter optimization: 5S ribosome RNA (Eubacteria subfamily), group I intron (IC1 subfamily), tmRNA, and tRNA; benchmarking: small subunit ribosomal RNA (Alphaproteobacteria subfamily), SRP RNA (Protozoan subfamily), RNase P RNA (A bacteria subfamily) and telomerase RNA). Specific sequences lists are provided in "TurboFoldII_SelectedSequenceList.zip".

Section 5. Performance of alignment methods on benchmarking dataset

Table S1. Alignment prediction sensitivity and PPV for each method on each dataset:

	small subunit rRNA					
	5 sequences		10 sequences		20 sequences	
	sensitivity	PPV	sensitivity	PPV	sensitivity	PPV
TurboFold II	0.949	0.961	0.957	0.968	0.956	0.968
Clustal Omega	**0.972**	**0.982**	**0.975**	**0.983**	**0.973**	**0.982**
ClustalW	0.912	0.911	0.910	0.909	0.906	0.906
LocARNA	0.905	0.912	0.876	0.880	0.811	0.816
MXSCARNA	0.902	0.914	0.929	0.941	0.932	0.945
ProbCons	0.922	0.933	0.939	0.948	0.943	0.953
MAFFT	0.942	0.951	0.956	0.964	0.960	0.969
R-Coffee	0.934	0.949	0.951	0.964	0.951	0.965

	RNase P					
	5 sequences		10 sequences		20 sequences	
	sensitivity	PPV	sensitivity	PPV	sensitivity	PPV
TurboFold II	**0.830**	**0.799**	**0.839**	**0.814**	**0.839**	**0.819**
Clustal Omega	0.805	0.783	0.814	0.800	0.811	0.804
ClustalW	0.563	0.534	0.581	0.558	0.567	0.554
LocARNA	0.643	0.610	0.612	0.588	0.597	0.580
MXSCARNA	0.745	0.729	0.772	0.762	0.786	0.780
ProbCons	0.764	0.732	0.782	0.760	0.794	0.778
MAFFT	0.794	0.767	0.818	0.796	0.828	0.806
R-Coffee	0.582	0.567	0.624	0.618	0.618	0.623

	SRP RNA					
	5 sequences		10 sequences		20 sequences	
	sensitivity	PPV	sensitivity	PPV	sensitivity	PPV
TurboFold II	0.498	0.471	0.517	0.497	0.533	0.514
Clustal Omega	0.404	0.407	0.413	0.422	0.423	0.434
ClustalW	0.411	0.384	0.401	0.377	0.404	0.381
LocARNA	**0.516**	0.483	0.513	0.485	0.515	0.489
MXSCARNA	0.415	0.411	0.427	0.434	0.435	0.448
ProbCons	0.422	0.402	0.444	0.431	0.467	0.453
MAFFT	0.505	**0.486**	**0.540**	**0.522**	**0.563**	**0.544**
R-Coffee	0.427	0.413	0.453	0.447	0.473	0.475

	telomerase					
	5 sequences		10 sequences		20 sequences	
	sensitivity	PPV	sensitivity	PPV	sensitivity	PPV
TurboFold II	**0.654**	**0.589**	**0.664**	**0.604**	**0.670**	**0.610**
Clustal Omega	0.642	0.585	0.647	0.596	0.642	0.598
ClustalW	0.574	0.508	0.571	0.509	0.568	0.510
LocARNA	0.522	0.462	0.524	0.467	0.523	0.468
MXSCARNA	0.596	0.549	0.600	0.560	0.605	0.569
ProbCons	0.621	0.555	0.631	0.572	0.640	0.582
MAFFT	0.632	0.571	0.649	0.589	0.660	0.600
R-Coffee	0.618	0.572	0.623	0.589	0.626	0.605

Table S2: Structure prediction sensitivity and PPV for each method on each dataset:

	small subunit rRNA					
	5 sequences		10 sequences		20 sequences	
	sensitivity	PPV	sensitivity	PPV	sensitivity	PPV
TurboFold II	0.632	0.648	0.645	0.658	0.649	0.657
LocARNA	0.605	0.582	0.622	0.605	0.604	0.589
MaxExpect	0.499	0.495	0.505	0.503	0.501	0.498
MXSCARNA	**0.644**	**0.673**	**0.670**	**0.705**	**0.680**	**0.718**
RNAalifold (Clustal Omega)	0.588	0.644	0.605	0.686	0.586	0.706
RNAalifold (ClustalW)	0.544	0.603	0.542	0.650	0.498	0.680
RNAalifold (MAFFT)	0.545	0.607	0.580	0.668	0.548	0.693
TurboFold	0.623	0.637	0.641	0.651	0.648	0.652

	RNase P					
	5 sequences		10 sequences		20 sequences	
	sensitivity	PPV	sensitivity	PPV	sensitivity	PPV
TurboFold II	**0.792**	**0.754**	**0.815**	0.761	**0.823**	0.763
LocARNA	0.612	0.587	0.554	0.600	0.505	0.614
MaxExpect	0.652	0.583	0.654	0.586	0.653	0.582
MXSCARNA	0.656	0.649	0.642	0.679	0.630	0.687
RNAalifold (Clustal Omega)	0.514	0.666	0.490	0.718	0.431	0.743
RNAalifold (ClustalW)	0.452	0.580	0.400	0.662	0.333	0.725
RNAalifold (MAFFT)	0.614	0.704	0.565	0.751	0.510	**0.776**
TurboFold	0.780	0.750	0.808	**0.762**	0.815	0.762

	SRP					
	5 sequences		10 sequences		20 sequences	
	sensitivity	PPV	sensitivity	PPV	sensitivity	PPV
TurboFold II	**0.752**	0.823	**0.762**	0.828	**0.767**	0.831
LocARNA	0.713	0.732	0.674	0.748	0.616	0.772
MaxExpect	0.665	0.682	0.654	0.671	0.657	0.674
MXSCARNA	0.707	0.703	0.714	0.722	0.725	0.736
RNAalifold (Clustal Omega)	0.192	0.671	0.125	**0.887**	0.110	**0.922**
RNAalifold (ClustalW)	0.273	0.552	0.147	0.797	0.103	0.908
RNAalifold (MAFFT)	0.490	0.771	0.383	0.863	0.320	0.894
TurboFold	0.736	**0.824**	0.751	0.832	0.760	0.838

	telomerase					
	5 sequences		10 sequences		20 sequences	
	sensitivity	PPV	sensitivity	PPV	sensitivity	PPV
TurboFold II	**0.825**	0.679	**0.833**	0.683	**0.835**	0.681
LocARNA	0.616	0.510	0.611	0.571	0.581	0.615
MaxExpect	0.581	0.433	0.582	0.434	0.583	0.434
MXSCARNA	0.652	0.538	0.703	0.608	0.712	0.625
RNAalifold (Clustal Omega)	0.489	0.539	0.357	0.662	0.268	**0.811**
RNAalifold (ClustalW)	0.447	0.452	0.300	0.555	0.223	0.721
RNAalifold (MAFFT)	0.599	0.584	0.468	0.671	0.368	0.776
TurboFold	0.824	**0.683**	0.831	**0.684**	0.832	0.681

Section 6. Performance of alignment methods on BRAliBase 2.0

The benchmark of TurboFold II on BRAliBase 2.0 (11) is provided here. BraliBase 2.0 includes segments of group II introns, rRNA, SRP RNA, tRNA, and U5 RNA. The Sum of Pairs Score (SPS) is the ratio between the total number of columns in which nucleotide pairs or nucleotides aligned to a gap are identically aligned in dataset. The benchmark results of other methods are from the BRAliBase website: http://projects.binf.ku.dk/pgardner/bralibase/bralibase2.html.

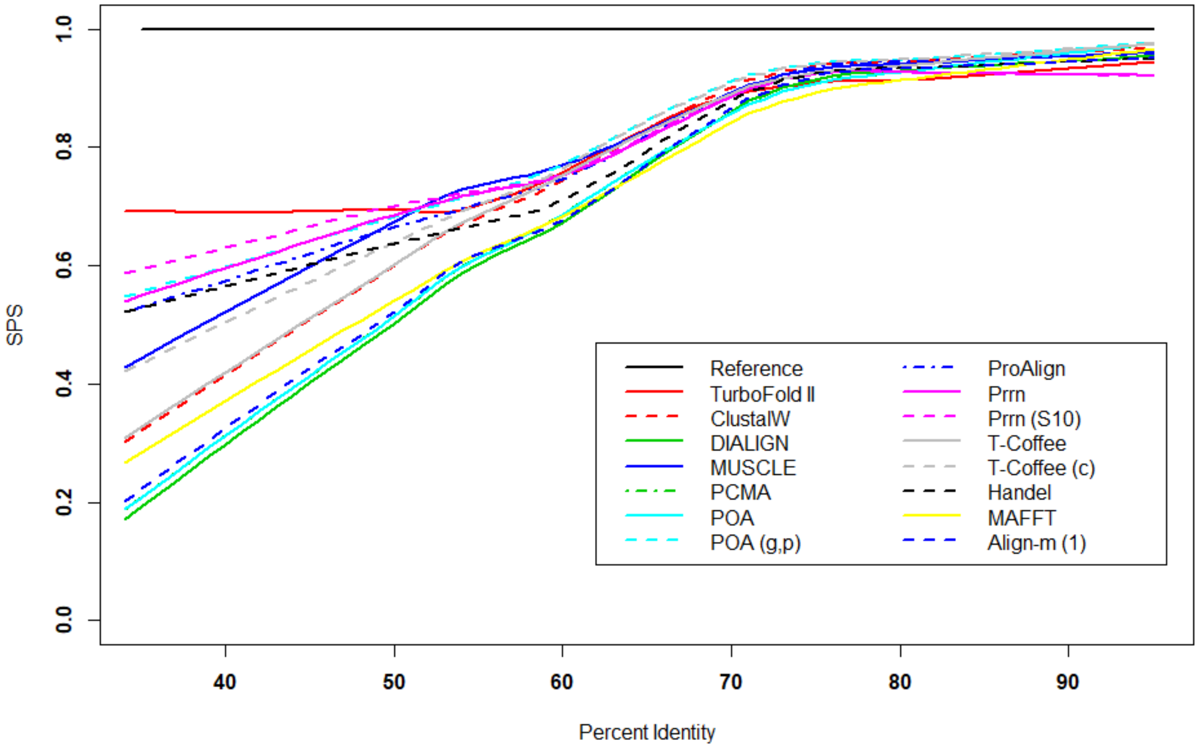
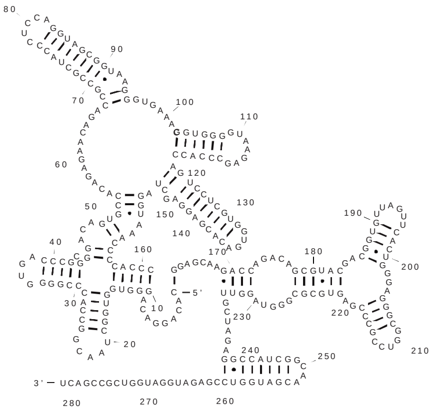


Figure S3. **The plot shows the alignment accuracy as function of the sequence identity.** The SPSs for methods other than TurboFold II are obtained from the supplementary data for BRAliBase 2.0 (11). The trend lines are plotted using LOWESS (locally weighted scatterplot smoothing) in R with the smoother span set to 0.5.

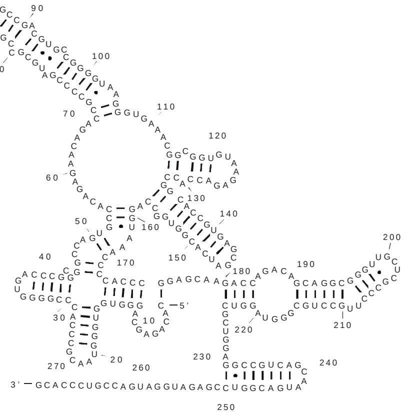
Section 6. Known and predicted structures and alignments for RNase P RNA from *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26, Mycobacterium tuberculosis, and Lake Griffy A #8*.

Figure S4 show the database structures for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis,* and *Lake Griffy A #8* from the RNase P database (6). Figure S5 shows the database alignments for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*. Figure S6-S11 show the predicted sequence alignments by TurboFold II, Clustal Omega, ClustalW, LocARNA and MXSCARNA and ProbCons, respectively, for this set of H=5 sequences.

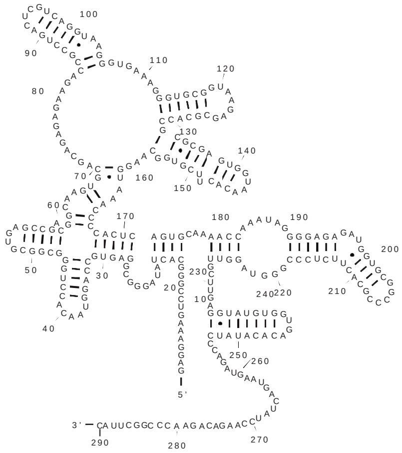
Figure S12-S18 show the predicted structures by TurboFold II, LocARNA, MaxExpect, MXSCARNA, RNAalifold (using Clustal Omega alignment), RNAalifold (using ClustalW alignment), and TurboFold, respectively, for this set of H=5 sequences. For LocARNA, RNAalifold, and MXSCARNA, which predict consensus structure for multiple sequences, to avoid the inconsistency between the consensus structure and each sequence, the predicted structures for each sequence were generated by removing base pairing between nucleotide and insertion/deletion (gap) positions.



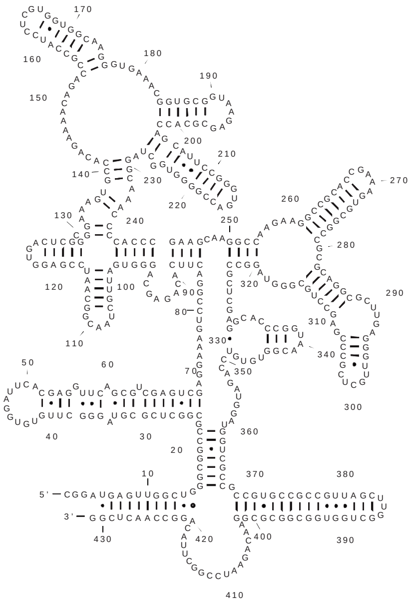
(a) *Nocardioides albus*



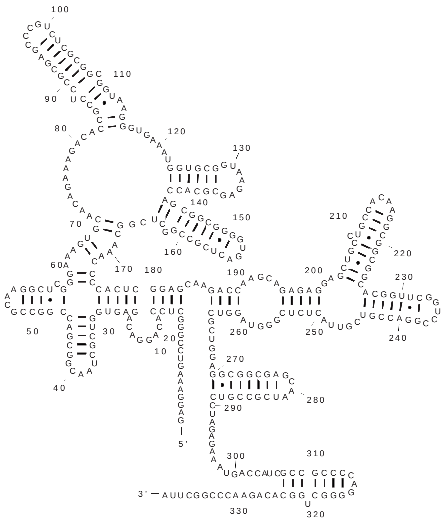
(b) *Propioniferax innocua*



(c) *Salt Marsh A26*



(d) *Mycobacterium tuberculosis*



(e) *Lake Griffy A #8*

Figure S4. **Known structures for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.** Known structures for *Nocardioides albus* (a), *Propioniferax innocua* (b), *Salt Marsh A26* (c), *Mycobacterium tuberculosis* (d) and *Lake Griffy A #8* (e) from the RNase P database (6). 5’ and 3’ ends of sequences are indicated by “5’” and “3’”. A heavy line between two nucleotides indicates the base pairing interaction between the nucleotides.

```
N.albus             ------------------------------------------------------------
SM-A26              ------------------------------------------------------------
M.tuberculosis      CGGAUGAGUUGGCUGGGCGGCCGCGGCUCGCGUAGGGCUUGUGUGGAUUCACGAGGUUCA
LGA8                ------------------------------------------------------------
P.innocua           ------------------------------------------------------------

N.albus             ---------------------------CACAGGACAGGGUGGUGGCUAACGGCCACCCGG
SM-A26              ----------gaggaaagucCGGGCACUAUAGGGCGGAGUGCCAGGUAACACCUGGGCGG
M.tuberculosis      GCGUCGAGUCGAGGAAAGUCCGGACUUCACAGAGCAGGGUGAUUGCUAACGGCAAUCCGA
LGA8                ----------gaggaaaguccgggcUCCACAGGACAGAGUGGUCGCUAACGGCGACCGGC
P.innocua           ---------------------------CACAGAGCAGGGUGGUGGGUAACGCCCACCCGG

N.albus             GGUGACCCGCGGGACAGUGCCACAGAGAACAGA-CCGCCGCUACC------CUCCA----
SM-A26              CGUGAGCCGACGGCAAGUGCAGCAGAGAGAAG-ACCGCCUGA----------CUCG----
M.tuberculosis      GGUGACUCGCGGGAAAGUGCCACAGAAAACAG-ACCGCCAUC---------CUCGU----
LGA8                CGCAAGGCUCGGGAAAGUGCAACAGAAAGAC--ACCGCCUCCGCGAG----CCCGU----
P.innocua           GGUGACCCGCGGGCCAGUGCCACAGAGAACAGA-CCGCCCCGAUGCGCCGG-UUCGCCGA

N.albus             --GGUAGCGGUAAGGGUGAAACGGUGGGGUAAGAGCCCACCAGUCCUCGUGGUGACAC-G
SM-A26              -----UCAGGUAAGGGUGAAAGGGUGCGGUAAGAGCGCACC-GCGCGAGUGGUAACACUU
M.tuberculosis      -----GGUGGCAAGGGUGAAACGGUGCGGUAAGAGCGCACCAGCAUUCCGGGUGACCG-G
LGA8                CUCGCGGCGGUAAGGGUGAAAUGGUGCGGUAAGAGCGCACCAGCGGCGGG-GUGA-CU-C
P.innocua           CGUGCCGGGGUAAGGGUGAAACGGCGGUGUAAGAGACCACCGGCACCGUGAGCGAUCA-C

N.albus             AGGAGCU-AGGUAAACCCCACCCGGAGCAAGACCAGAC----------------------
SM-A26              CGUGGC-AAGGUAAACCCCACUCAGUGCAAAACCAAAU----------------------
M.tuberculosis      GGUGGCU-AGGCAAACCCCACCCGAAGCAAGGCCAAGAAGGCCGCACCGAAAGUGCGGCC
LGA8                GCCGGCU-CGGCAAACCCCACUCGGAGCAAGACCAAG-----------------------
P.innocua           GGUGGCC-AGGUAAACCCCACCCGGAGCAAGACCAGAC----------------------

N.albus             -AGCGUACGACGGUG-----UUAGUU----CACUGGG--AGGGCGGUCCGCCCGA-----
SM-A26              AGGGGAGAGA--------------------------U--GGUGCGGCCCGCACU------
M.tuberculosis      GCGCAGGCGC-------------------------UU-GAGGGUUGCUCGCCCGA-----
LGA8                CAGAGAG-GAGCUGCCUGCC-ACAA-GGCGGCGGC--ACGGUUCGGUCCGGACCGUCGUU
P.innocua           -AGCAGGC--------------------------------GGGUUGCUCGCCC------U

N.albus             -GUGCGCGGGUAGGUUGCUAGAGGCCAUCGGCAACGAUGGUCCGAGAUGGAUGGUCGCCG
SM-A26              -UCUCCCGGGUAGGUUGCUUGAGGUAUGUGGUGACACAUAUCCCAGAUGAAUGACUAUCC
M.tuberculosis      -GCCUGCGGGUAGGCCGCUCGAGGCACCCGGUAACGGUGUGUCCAGAUGGAUGGUCGCC-
LGA8                A-CUCUCGGGUAGGUCGCUGGAGGCGGCGAGCAAUCGCCGUCCUAGAGAAAUGACCAUC-
P.innocua           UGCCUGCGGGUAGGUCGCUGGAGGCCGUCAGCAAUGACGGUCCGAGAUGGAUGACCGUCC

N.albus             A----------------------------------------CU-----------------
SM-A26              A----------------------------------------AGacagaacccggcuuac-
M.tuberculosis      --GCCGUGCCGCCGUUAGCUUG-GCUgGUGGCGGCGC-GGA--ACAGAAUCCGGCUUACA
LGA8                --GCCGCCC-----------CAG----------GGGCUGGC--acagaacccggcuua--
P.innocua           CA---------------------------------------CG-----------------

N.albus             --------------
SM-A26              --------------
M.tuberculosis      GGCCAACUCGUCCG
LGA8                --------------
P.innocua           --------------
```

Figure S5. **Database alignments for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

```
N.albus             ------------------------------------------------------------
SM-A26(42)          -GA---------------------------------------------------------
M.tuberculosis      CGGAUGAGUUGGCUGGGCGGCCGCGGCUCGCGUAGGGCUUGUGUGGAUUCACGAGGUUCA
LGA8                GA----------------------------------------------------------
P.innocua           ------------------------------------------------------------

N.albus             ----------------------------CACAGGACAGGGUGGUGGCUAACGGCCACCCG
SM-A26(42)          ------------GGAAAGUCCGGGCACUAU-AGGGCGGAGUGCCAGGUAACACCUGGGCG
M.tuberculosis      GCGUCGAGUCGAGGAAAGUCCGGACUUCAC-AGAGCAGGGUGAUUGCUAACGGCAAUCCG
LGA8                ------------GGAAAGUCCGGGCUCCAC-AGGACAGAGUGGUCGCUAACGGCGACCGG
P.innocua           --------------------------CAC--AGAGCAGGGUGGUGGGUAACGCCCACCCG

N.albus             GGGUGACCCGCGGGACAGUGCCACAGAGAACAGACCGCCGCUAC------CCUCCA----
SM-A26(42)          GCGUGAGCCGACGGCAAGUGCAGCAGAGAGAAGACCGCCUG---------ACUCGU----
M.tuberculosis      AGGUGACUCGCGGGAAAGUGCCACAGAAAACAGACCGCCAU---------CCUCGU----
LGA8                CCGCAAGGCUCGGGAAAGUGCAACAGAAA-GACACCGCCUCCGC----GAGCCCGU---C
P.innocua           GGGUGACCCGCGGGCCAGUGCCACAGAGAACAGACCGCCCCGAUGCGCCGGUUCGCCGAC

N.albus             -GGUAGCGGUAAGGGUGAAACGGUGGGGUAAGAGCCCACCAGUCCUCGUGGUGACAC-GA
SM-A26(42)          -----CAGGUAAGGGUGAAAGGGUGCGGUAAGAGCGCACCGCGCG-AGUGGUAACACUUC
M.tuberculosis      ----GGUGGCAAGGGUGAAACGGUGCGGUAAGAGCGCACCAGCAUUCCGGGUGACCG-GG
LGA8                UCGCGGCGGUAAGGGUGAAAUGGUGCGGUAAGAGCGCACCAGCGG-CGGGGUGACUC--G
P.innocua           GUGCCGGGGUAAGGGUGAAACGGCGGUGUAAGAGACCACCGGCACCGUGAGCGAUCA-CG

N.albus             GGAGCUAGGUAAACCCCACCCGGAGCAAGACCAGACAGCGUACGACG----GUGUUAGUU
SM-A26(42)          GUGGCAAGGUAAACCCCACUCAGUGCAAAACCAAAUAGGGGAGAG---------------
M.tuberculosis      GUGGCUAGGCAAACCCCACCCGAAGCAAGGCCAAGAAGGCCGCA-------CCGAAAGUG
LGA8                CCGGCUCGGCAAACCCCACUCGGAGCAAGACCAAGCAGAGAGGAGCUGCCUGCCACAAGG
P.innocua           GUGGCCAGGUAAACCCCACCCGGAGCAAGACCAGACAGCAGGC-----------------

N.albus             CACUGGGA-----------GGGCGGUCCGCCC-----GAGUGCGCGGGUAGGUUGCUAGA
SM-A26(42)          -------AUG---------GUGCGGCCCGCAC-----U-UCUCCCGGGUAGGUUGCUUGA
M.tuberculosis      CGGCCGCGCAGGCGCUUGAGGGUUGCUCGCCC-----GAGCCUGCGGGUAGGCCGCUCGA
LGA8                CGGCGGCACG---------GUUCGGUCCGGACCGUCGUUACUCUCGGGUAGGUCGCUGGA
P.innocua           -------------------GGGUUGCUCGCCC-----UUGCCUGCGGGUAGGUCGCUGGA

N.albus             GGCCAUCGGCAACGAUGGUCCGAGAUGGAUGGUCGCCG----------------------
SM-A26(42)          GGUAUGUGGUGACACAUAUCCCAGAUGAAUGACUAUCC----------------------
M.tuberculosis      GGCACCCGGUAACGGUGUGUCCAGAUGGAUGGUCGCCGCCGUGCCGCCGUUAGCUUGGCU
LGA8                GGCGGCGAGCAAUCGCCGUCCUAGAGAAAUGACCAUCGCCGCCCC----------AG---
P.innocua           GGCCGUCAGCAAUGACGGUCCGAGAUGGAUGACCGUCC----------------------

N.albus             ----------------A--------------------------CU
SM-A26(42)          -----------AAGACAGAACCCGGCUU---------------AC
M.tuberculosis      GGUGGCGGCGCGGAACAGAAUCCGGCUUACAGGCCAACUCGUCCG
LGA8                ------GGGCUGGCACAGAACCCGGCUU----------------A
P.innocua           ---------------CA--------------------------CG
```

Figure S6. **Predicted alignments by TurboFold II for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

```
N.albus             ------------------------------------------------------------
SM-A26              ------------------------------------------------------------
M.tuberculosis      CGGAUGAGUUGGCUGGGCGGCCGCGGCUCGCGUAGGGCUUGUGUGGAUUCACGAGGUUCA
LGA8                ------------------------------------------------------------
P.innocua           ------------------------------------------------------------

N.albus             ---------------------------CACAGGACAGGGUGGUGGCUAACGGCCACCCGG
SM-A26              ----------GAGGAAAGUCCGGGCACUAUAGGGCGGAGUGCCAGGUAACACCUGGGCGG
M.tuberculosis      GCGUCGAGUCGAGGAAAGUCCGGACUUCACAGAGCAGGGUGAUUGCUAACGGCAAUCCGA
LGA8                ----------GAGGAAAGUCCGGGCUCCACAGGACAGAGUGGUCGCUAACGGCGACCGGC
P.innocua           ---------------------------CACAGAGCAGGGUGGUGGGUAACGCCCACCCGG

N.albus             GGUGACCCGCGGGACAGUGCCACAGAGAACAGACCGCCGCUACCCUCCAGGU--------
SM-A26              CGUGAGCCGACGGCAAGUGCAGCAGAGAGAAGACCGCCUGACUC---G------------
M.tuberculosis      GGUGACUCGCGGGAAAGUGCCACAGAAAACAGACCGCC---AUCCUCGU-----------
LGA8                CGCAAGGCUCGGGAAAGUGCAACAGAAA-GACACCGCCUCCGC----G---AGCCCGUCU
P.innocua           GGUGACCCGCGGGCCAGUGCCACAGAGAACAGACCGCCCCGAUGCGCCGGUUCGCCGACG

N.albus             ---AGCGGUAAGGGUGAAACGGUGGGGUAAGAGCCCACCAGUCCUCGUGGUGACAC-GAG
SM-A26              ---UCAGGUAAGGGUGAAAGGGUGCGGUAAGAGCGCACCGCGCGAG-UGGUAACACUUCG
M.tuberculosis      ---GGUGGCAAGGGUGAAACGGUGCGGUAAGAGCGCACCAGCAUUCCGGGUGACCG-GGG
LGA8                CGCGGCGGUAAGGGUGAAAUGGUGCGGUAAGAGCGCACCAGCGGCG-GGGUGACUC--GC
P.innocua           UGCCGGGGUAAGGGUGAAACGGCGGUGUAAGAGACCACCGGCACCGUGAGCGAUCA-CGG

N.albus             GAGCUAGGUAAACCCCACCCGGAGCAAGACCAGACAGCGUAC----------GACGGUGU
SM-A26              UGGCAAGGUAAACCCCACUCAGUGCAAAACCAAAUAGGGGAGAGAUG-------------
M.tuberculosis      UGGCUAGGCAAACCCCACCCGAAGCAAGGCCAAGAAGGCCGCACC-------GAAAGUGC
LGA8                CGGCUCGGCAAACCCCACUCGGAGCAAGACCAAGCAGAGAGGAGCUGCCUGCCACAAGGC
P.innocua           UGGCCAGGUAAACCCCACCCGGAGCAAGACCAGACAGCAGGC----------G-------

N.albus             UAGUUCA-----CUGGGAGGGCGGUCCGCCCGAGUGCGCGGGUAGGUUGCUAGAGGCCAU
SM-A26              ------------------GUGCGGCCCGCACUU-CUCCCGGGUAGGUUGCUUGAGGUAUG
M.tuberculosis      GGCCGCGCAGGCGCUUGAGGGUUGCUCGCCCGAGCCUGCGGGUAGGCCGCUCGAGGCACC
LGA8                GGCGGCACGG----UUCGGUCCGGACCGUCGUUACUCUCGGGUAGGUCGCUGGAGGCGGC
P.innocua           -------------------GGUUGCUCGCCCUUGCCUGCGGGUAGGUCGCUGGAGGCCGU

N.albus             CGGCAACGAUGGUCCGAGAUGGAUGGUCGCCGACU-------------------------
SM-A26              UGGUGACACAUAUCCCAGAUGAAUGACUAUCCA---------------------------
M.tuberculosis      CGGUAACGGUGUGUCCAGAUGGAUGGUCGCCGCCGUGCCGCCGUUAGCUUGGCUGGUGGC
LGA8                GAGCAAUCGCCGUCCUAGAGAAAUGACCAUCGCCGCCCAG--------------------
P.innocua           CAGCAAUGACGGUCCGAGAUGGAUGACCGUCCCACG------------------------

N.albus             ---------------------------------------
SM-A26              ------AGACAGAACCCGGCUUAC---------------
M.tuberculosis      GGCGCGGAACAGAAUCCGGCUUACAGGCCAACUCGUCCG
LGA8                GGGCUGGCACAGAACCCGGCUUA----------------
P.innocua           ---------------------------------------
```

Figure S7. **Predicted alignments by Clustal Omega for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

```
N.albus             ------------------------------------------------------------
M.tuberculosis      CGGAUGAGUUGGCUGGGCGGCCGCGGCUCGCGUAGGGCUUGUGUGGAUUCACGAGGUUCA
P.innocua           ------------------------------------------------------------
SM-A26              ------------------------------------------------------------
LGA8                ------------------------------------------------------------

N.albus             ---------------------------CACAGGACAGGGUGGUGGCUAACGGCCACCCGG
M.tuberculosis      GCGUCGAGUCGAGGAAAGUCCGGACUUCACAGAGCAGGGUGAUUGCUAACGGCAAUCCGA
P.innocua           ---------------------------CACAGAGCAGGGUGGUGGGUAACGCCCACCCGG
SM-A26              ----------GAGGAAAGUCCGGGCACUAUAGGGCGGAGUGCCAGGUAACACCUGGGCGG
LGA8                ----------GAGGAAAGUCCGGGCUCCACAGGACAGAGUGGUCGCUAACGGCGACCGGC

N.albus             GGUGACCCGCGGGACAGUGCCACAGAGAACAGACCGCCGCUACCCUCCAG----------
M.tuberculosis      GGUGACUCGCGGGAAAGUGCCACAGAAAACAGACCGCC---AUCCUC-------------
P.innocua           GGUGACCCGCGGGCCAGUGCCACAGAGAACAGACCGCCCCGAUGCGCCGGUUCGCCGACG
SM-A26              CGUGAGCCGACGGCAAGUGCAGCAGAGAGAAGACCGCCU-----GACUCGUCA-------
LGA8                CGCAAGGCUCGGGAAAGUGCAACAGAAAGAC-ACCGCCUCCGCGAGCCGUCUC-------

N.albus             -GUAGCGGUAAGGGUGAAACGGUGGGGUAAGAGCCCACCAGUCCUCGUGGUGACACGAGG
M.tuberculosis      -GUGGUGGCAAGGGUGAAACGGUGCGGUAAGAGCGCACCAGCAUUCCGGGUGACCGGGGU
P.innocua           UGCCGGGGUAAGGGUGAAACGGCGGUGUAAGAGACCACCGGCACCGUGAGCGAUCACGGU
SM-A26              ------GGUAAGGGUGAAAGGGUGCGGUAAGAGCGCACCGCGCGAGUGGUAACACUUCGU
LGA8                -GCGGCGGUAAGGGUGAAAUGGUGCGGUAAGAGCGCACCAGCGGCGGGGUGACUCGCCG-

N.albus             AGCUAGGUAAACCCCACCCGGAGCAAGACCAGACAG--CGUAC-GACGGUGUUAGUUCAC
M.tuberculosis      GGCUAGGCAAACCCCACCCGAAGCAAGGCCAAGAAGGCCGCACCGAAAGUGCGGCCGCGC
P.innocua           GGCCAGGUAAACCCCACCCGGAGCAAGACCAGACAG-----------------------C
SM-A26              GGCAAGGUAAACCCCACUCAGUGCAAAACCAAAUAGG--GGAGAG---------------
LGA8                -GCUCGGCAAACCCCACUCGGAGCAAGACCAAGCAGA--GAGGAGCUGCCUGCCACAAGG

N.albus             UGG-----GAGGGCGGUCCGCCC-----GAGUGCGCGGGUAGGUUGCUAGAGGCCAUCGG
M.tuberculosis      AGGCGCUUGAGGGUUGCUCGCCC-----GAGCCUGCGGGUAGGCCGCUCGAGGCACCCGG
P.innocua           AGGC------GGGUUGCUCGCCC-----UUGCCUGCGGGUAGGUCGCUGGAGGCCGUCAG
SM-A26              -------AUGGUGCGGCCCGCAC------UUCUCCCGGGUAGGUUGCUUGAGGUAUGUGG
LGA8                CGGCGGCACGGUUCGGUCCGGACCGUCGUUACUCUCGGGUAGGUCGCUGGAGGCGGCGAG

N.albus             CAACGAUGGUCCGAGAUGGAUGGUCGCCGACU----------------------------
M.tuberculosis      UAACGGUGUGUCCAGAUGGAUGGUCGCCGCCGUGCCGCCGUUAGCUUGGCUGGUGGCGGC
P.innocua           CAAUGACGGUCCGAGAUGGAUGACCGUC-CCACG--------------------------
SM-A26              UGACACAUAUCCCAGAUGAAUGACUAUC-------CAAG-------ACAGAACCCGGCUU
LGA8                CAAUCGCCGUCCUAGAGAAAUGACCAUCGCCGCCCCAGGGGCUGGCACAGAACCCGGCUU

N.albus             ------------------------------------
M.tuberculosis      GCGGAACAGAAUCCGGCUUACAGGCCAACUCGUCCG
P.innocua           ------------------------------------
SM-A26              AC----------------------------------
LGA8                A-----------------------------------
```

Figure S8. **Predicted alignments by ClustalW for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

```
SM-A26              GAGGAAAGUCCGGGCACUAUAG--GGCGGAGU--GCCAGG--------UAACACCUGGGC
LGA8                GAGGAAAGUCCGGGCUCCACAG--GACAGAGU--GGUCGC--------UAACGGCGACCG
N.albus             -----------------CACAG--GACAGGGU--GGUGGC--------UAACGGCCACCC
P.innocua           -----------------CACAG--AGCAGGGU--GGUGGG--------UAACGCCCACCC
M.tuberculosis      CGGAUGAGUUGGCUGGGCGGCCGCGGCUCGCGUAGGGCUUGUGUGGAUUCACGAGGUUCA

SM-A26              GGCGUGAGCC---GACGGCAAGUGCAGCAGAGAG-AA-------GAC----CGCCUGAC-
LGA8                GCCGCAAGGC---UCGGGAAAGUGCAACAGAAAG-AC-------ACCGCCUCCGCGAGC-
N.albus             GGGGUGACCC---GCGGGACAGUGCCACAGAGAACAG-------ACCGCCGCUACCCUC-
P.innocua           GGGGUGACCC---GCGGGCCAGUGCCACAGAGAACAG-------ACCGCCCCGAUGCGCC
M.tuberculosis      GCGUCGAGUCGAGGAAAGUCCGGACUUCACAGAGCAGGGUGAUUGCUAACGGCAAUCCGA

SM-A26              ------UCG-UCA---GGU---------------------------------AAGGGUGA
LGA8                ------CCGUCUC---GCGGC----------------------------GGUAAGGGUGA
N.albus             ----------CAG---GUAGC----------------------------GGUAAGGGUGA
P.innocua           GGUUCGCCGACGU---GCCGG----------------------------GGUAAGGGUGA
M.tuberculosis      GGUGACUCGCGGGAAAGUGCCACAGAAAACAGACCGCCAUCCUCGUGGUGGCAAGGGUGA

SM-A26              AAGGGUGCGGUAAGAGCGCACCGCGCG-AGUGGUAACACUUCGUGGCAAGGUAAACCCCA
LGA8                AAUGGUGCGGUAAGAGCGCACCAGCGG-CGGGGUGACUCG-CCGGCU-CGGCAAACCCCA
N.albus             AACGGUGGGGUAAGAGCCCACCAGUCCUCGUGGUGACACGAGGAGCU-AGGUAAACCCCA
P.innocua           AACGGCGGUGUAAGAGACCACCGGCACCGUGAGCGAUCACGGUGGCC-AGGUAAACCCCA
M.tuberculosis      AACGGUGCGGUAAGAGCGCACCAGCAUUCCGGGUGACCGGGGUGGCU-AGGCAAACCCCA

SM-A26              CUCAGUGCAAAACCAAAUAGGGGAGAG----------------------AUGGUGCGGCC
LGA8                CUCGGAGCAAGACCAAGCAGAGAGGAGCUGCCUGCCACAAGGCGGCGGCACGGUUCGGUC
N.albus             CCCGGAGCAAGACCAGACAGCGUACGACGGUGUU-----AGUUCACUG-GGAGGGCGGUC
P.innocua           CCCGGAGCAAGACCAGACAGCAGGC---------------------------GGGUUGCU
M.tuberculosis      CCCGAAGCAAGGCCAAGAAGGCCGCACCGAAAGUGCGGCCGCGCAGGC-GCUUGAGGGUU

SM-A26              CGCACU-----UCUCCC-GGGUAGGUUGCUUGAGGUAUGUGGUGACAC-AUAUCCCAGAU
LGA8                CGGACCGUCGUUACUCUCGGGUAGGUCGCUGGAGGCGGCGAGCAAUCG-CCGUCCUAGAG
N.albus             CGCCCG-----AGUGCGCGGGUAGGUUGCUAGAGGCCAUCGGCAACGA-UGGUCCGAGAU
P.innocua           CGCCCU-----UGCCUGCGGGUAGGUCGCUGGAGGCCGUCAGCAAUGA-CGGUCCGAGAU
M.tuberculosis      GCUCGCCCGAGCCUGC--GGGUAGGCCGCUCGAGGCACCCGGUAACGGUGUGUCC-AGAU

SM-A26              GAAUGACUAU----------------CCAAG-----------------ACAGAACCCGGC
LGA8                AAAUGACCAUCGCC----------GCCCCAGGGGC---------UGGCACAGAACCCGGC
N.albus             GGAUGGUCG----------------------------------------------CCGAC
P.innocua           GGAUGACCGU---------------------------------------------CCCAC
M.tuberculosis      GGAUGGUCGCCGCCGUGCCGCCGUUAGCUUGGCUGGUGGCGGCGGAACAGAAUCCGGC

SM-A26              UUAC---------------
LGA8                UUA----------------
N.albus             U------------------
P.innocua           G------------------
M.tuberculosis      UUACAGGCCAACUCGUCCG
```

Figure S9. **Predicted alignments by LocARNA for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

```
N.albus            CACA--------------------------------------------------------
SM-A26             GA----------------------------------------------------------
M.tuberculosis     CGGAUGAGUUGGCUGGGCGGCCGCGGCUCGCGUAGGGCUUGUGUGGAUUCACGAGGUUCA
LGA8               GA----------------------------------------------------------
P.innocua          C-----------------------------------------------------------

N.albus            -------------------------------GGACAGGGUGGUGGCUAACGGCCACCCGG
SM-A26             ------------GGAAAGUCCGGGCACUAUAGGGCGGAGUGCCAGGUAACACCUGGGCGG
M.tuberculosis     GCGUCGAGUCGAGGAAAGUCCGGACUUCACAGAGCAGGGUGAUUGCUAACGGCAAUCCGA
LGA8               ------------GGAAAGUCCGGGCUCCACAGGACAGAGUGGUCGCUAACGGCGACCGGC
P.innocua          ----------------------------ACAGAGCAGGGUGGUGGGUAACGCCCACCCGG

N.albus            GGUGACCCGCGGGACAGUGCCACAGAGAACAGACCGCCGCU---AC--C----C---UCC
SM-A26             CGUGAGCCGACGGCAAGUGCAGCAGAGAGAAGACCGCC------UGACU----C---GUC
M.tuberculosis     GGUGACUCGCGGGAAAGUGCCACAGAAAACAGACCGCC------AU--C----C---UCG
LGA8               CGCAAGGCUCGGGAAAGUGCAACAGAAAG-ACACCGCCUCCGCGAG-CC----C---GUC
P.innocua          GGUGACCCGCGGGCCAGUGCCACAGAGAACAGACCGCCCCGAUGCG-CCGGUUCGCCGAC

N.albus            AGGUAGCGGUAAGGGUGAAACGGUGGGGUAAGAGCCCACCAGUCCUCG-UGGUGAC-ACG
SM-A26             ------AGGUAAGGGUGAAAGGGUGCGGUAAGAGCGCACCGCGCG-AG-UGGUAAC-ACU
M.tuberculosis     UG---GUGGCAAGGGUGAAACGGUGCGGUAAGAGCGCACCAGCAUUCC-GGGUGAC-CGG
LGA8               UCGCGGCGGUAAGGGUGAAAUGGUGCGGUAAGAGCGCACCAGCGG-CG-GGGUGAC-UCG
P.innocua          GUGCCGGGGUAAGGGUGAAACGGCGGUGUAAGAGACCACCGGCAC-CGUGAGCGAUCACG

N.albus            -AGGAGCUAGGUAAACCCCACCCGGAGCAAGACCAGACAGC--GUA-CG---ACGGUGUU
SM-A26             UCGUGGCAAGGUAAACCCCACUCAGUGCAAAACCAAAUAGG--GGAGAG-----------
M.tuberculosis     -GGUGGCUAGGCAAACCCCACCCGAAGCAAGGCCAAGAAGGCCGCACCG---AAAGUGCG
LGA8               --CCGGCUCGGCAAACCCCACUCGGAGCAAGACCAAGCAGA--GAGGAGCUGCCUGCCAC
P.innocua          --GUGGCCAGGUAAACCCCACCCGGAGCAAGACCAGACAGC--AGGCGG-----------

N.albus            AGUUC--ACUGG-----GAGG--GCGGUCCG-----CCCGAGUGCGCGGGUAGGUUGCUA
SM-A26             ------------------AUGGUGCGGCCCGCA------CUUCUCCCGGGUAGGUUGCUU
M.tuberculosis     GCCGC--GCAGGCGCUUGAGG--GUUGCUCG-----CCCGAGCCUGCGGGUAGGCCGCUC
LGA8               A--AGGCGGCGG-----CACGGUUCGGUCCGGACCGUCGUUACUCUCGGGUAGGUCGCUG
P.innocua          ---------------------G--UUGCUCG-----CCCUUGCCUGCGGGUAGGUCGCUG

N.albus            GAGGCCAUCGGCAACGAUGGUCCGAGAUGGAUGGUCGC----------------------
SM-A26             GAGGUAUGUGGUGACACAUAUCCCAGAUGAAUGACUAUC-------C------A------
M.tuberculosis     GAGGCACCCGGUAACGGUGUGUCCAGAUGGAUGGUCGCCGCCGUGCCGCCGUUAGCUUGG
LGA8               GAGGCGGCGAGCAAUCGCCGUCCUAGAGAAAUGACCAUCGCCGCCCC------AG--GGG
P.innocua          GAGGCCGUCAGCAAUGACGGUCCGAGAUGGAUGACCGUC-------C-------------

N.albus            ---------------------------C-G--------------ACU
SM-A26             --------------AGACAGAACCCGGCU---------------UAC
M.tuberculosis     CUGGUGGCGGCGCGGAACAGAAUCCGGCUUACAGGCCAACUCGUCCG
LGA8               C-----------UGGCACAGAACCCGGCU---------------U-A
P.innocua          ----------------------------C---------------ACG
```

Figure S10. **Predicted alignments by MXSCARNA for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

```
N.albus            --------------------------------------------------------
SM-A26             --------------------------------------------------------
M.tuberculosis     CGGAUGAGUUGGCUGGGCGGCCGCGGCUCGCGUAGGGCUUGUGUGGAUUCACGAGG
LGA8               --------------------------------------------------------
P.innocua          --------------------------------------------------------

N.albus            ---------------------------CAC---AGGACAGGGUGGUGGCUAACGGC
SM-A26             ----------GAGGAAAGUCCGGG---CACUAUAGGGCGGAGUGCCAGGUAACACC
M.tuberculosis     GCGUCGAGUCGAGGAAAGUCCGGACUUCAC---AGAGCAGGGUGAUUGCUAACGGC
LGA8               ----------GAGGAAAGUCCGGGCUCCAC---AGGACAGAGUGGUCGCUAACGGC
P.innocua          ---------------------------CAC---AGAGCAGGGUGGUGGGUAACGCC

N.albus            CCGGGGUGACCCG-------CGGGACAGUGCCACAGAGAACAGACCGCC--G--CU
SM-A26             GCGGCGUGAGCCG-------ACGGCAAGUGCAGCAGAGAGAAGACCGCC-UG----
M.tuberculosis     CCGAGGUGACUCG-------CGGGAAAGUGCCACAGAAAACAGACCGCCAUCC---
LGA8               CCGG------CCGCAAGGCUCGGGAAAGUGCAACAGAAAG-ACACCGCC-UCCGC-
P.innocua          CCGGGGUGACCCG-------CGGGCCAGUGCCACAGAGAACAGACCGCC--CCGAU

N.albus            -C--CUCCAG----GUAG--CGGUAAGGGUGAAACGGUGGGG--UAAGAGCCCACC
SM-A26             ----CUCGUC----------AGGUAAGGGUGAAAGGGUGCGG--UAAGAGCGCACC
M.tuberculosis     -----UCGUG----GUGG--C---AAGGGUGAAACGGUGCGG--UAAGAGCGCACC
LGA8               CCGUCUCG-C------GG--CGGUAAGGGUGAAAUGGUGCGG--UAAGAGCGCACC
P.innocua          CGGU-UCG-CCGACGUGCCGGGGUAAGGGUGAAACG--GCGGUGUAAGAGACCACC

N.albus            CU--CG--UGGUGAC-A--CGA--GG-AGCUA-GGUAAACCCCACCCGGAGCAAGA
SM-A26             G---CGAGUGGUAAC-----ACUUCGUGGC-AAGGUAAACCCCACUCAGUGCAAAA
M.tuberculosis     AUUCCG---GGUGAC----CGG--GGUGGCUA-GGCAAACCCCACCCGAAGCAAGG
LGA8               G--GCGG--GGUGAC---UCGC--C--GGCUC-GGCAAACCCCACUCGGAGCAAGA
P.innocua          A--CCG-----UGAGCGAUCAC--GGUGGC-CAGGUAAACCCCACCCGGAGCAAGA

N.albus            A-CAGC--------------GUACGACGGUGUUAGUUCACUGGGAGGGCG------
SM-A26             AUAGGGG-A----GAGAUG-GUG-------------------------CG------
M.tuberculosis     G-AAGGCCGCACCGAAA---GUGCGGCC-------------GCGCAGGCGCUUGAG
LGA8               G-CAGAGAG----GAGCUGCCUGCCAC-----------------AAGGCG------
P.innocua          A-CAGC---------------------------------------AGGCG------

N.albus            -C-CGCCC----------GAG------------UGCGCGGGUAGGUUGCUAGAG--
SM-A26             ----GCCC----------GCAC-----U---UCU-CCCGGGUAGGUUGCUUGAGGU
M.tuberculosis     GCUCGCCC----------GAGC----------CU--GCGGGUAGGCCGCUCGAG--
LGA8               -C-GGCACGGUUCGGUCCGGACCGUCGUUACUCU---CGGGUAGGUCGCUGGAG--
P.innocua          GCUCGCCC--U------UGC------------CU--GCGGGUAGGUCGCUGGAG--

N.albus            ----GCCAUC-GGCA---ACGAUG-GUCC--GAGAUGGAUGG---UCG--------
SM-A26             GGUGAC------------ACA-U--AUCC--CAGAUGAAUGACUAUCC--------
M.tuberculosis     ----GCAC-C---CGGUAACGGUGUGUCC---AGAUGGAUGG---UCGCCGCCGUG
LGA8               ----GCGG-CGAGCAAUCGCC----GUCCUAGAGA--AAUGACCAUCGCCGCC---
P.innocua          ----GCCGUC-AGCAAUGACG----GUCC--GAGAUGGAUGACCGUCC--------

N.albus            -----------------------------------CCGACU---------------
SM-A26             --------------------AAGACAGAAC-----CCGGCUUAC------------
M.tuberculosis     CGUUAGCUUGGCUGGUGGC-GGCGCGGAACAGAAUCCGGCUUACAGGCCAACUCGU
LGA8               -------------GG-GGCUGGCACAGAAC-----CCGGCUUA-------------
P.innocua          -----------------------------------ACG------------------
```

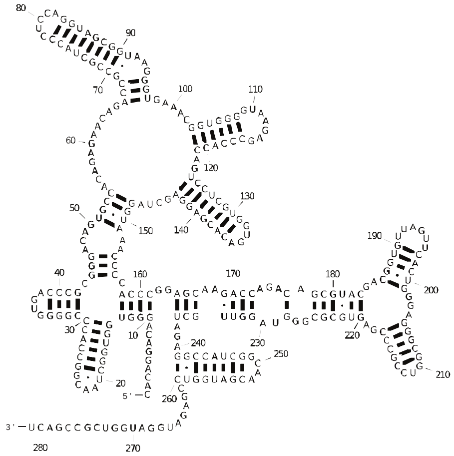
Figure S11. **Predicted alignments by ProbCons for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

```
N.albus             CACA-----------------------------------------------------
SM-A26(42)          GAGGAAAGU---CCGGGCACU------------------------------------
M.tuberculosis      CGGAUGAGUUGGCUGGGCGGCCGCGGCUCGCGUAGGGCUUGUGUGGAUUCACGAGGU
LGA8                GAGGAAAGU---CCGGGCUCC------------------------------------
P.innocua           CACA-----------------------------------------------------

N.albus             -------------------------------GGACAGGGUGGUGGCUAACGGCCACC
SM-A26(42)          ----------------------------AUAGGGCGGAGUGCCAGGUAACACCUGGG
M.tuberculosis      GCGUCGAGUCGAGGAAAGUCCGGACUUCACAGAGCAGGGUGAUUGCUAACGGCAAUC
LGA8                ----------------------------ACAGGACAGAGUGGUCGCUAACGGCGACC
P.innocua           -------------------------------GAGCAGGGUGGUGGGUAACGCCCACC

N.albus             GGUGACCCGCGGGACAGUGCCACAGAGAACAGACCGCCGCUACCCUC----------
SM-A26(42)          CGUGAGCCGACGGCAAGUGCAGCAGAGAGAAGACCGCC-UGA---------------
M.tuberculosis      GGUGACUCGCGGGAAAGUGCCACAGAAAACAGACCGCC---AUCC------------
LGA8                CGCAAGGCUCGGGAAAGUGCAACAGAAAG-ACACCGCCUCCGCGAGCCCGU------
P.innocua           GGUGACCCGCGGGCCAGUGCCACAGAGAACAGACCGCCCCGAUGCGCCGGUUCGCCG

N.albus             GGUAGCGGUAAGGGUGAAACGGUGGGGUAAGAGCCCACCAGUCCUCGUGGUGACACG
SM-A26(42)          CGUCA-GGUAAGGGUGAAAGGGUGCGGUAAGAGCGCACC-GCGCGAGUGGUAACACU
M.tuberculosis      CGUGGUGGCAAGGGUGAAACGGUGCGGUAAGAGCGCACCAGCAUUCCGGGUGACCGG
LGA8                CGCGGCGGUAAGGGUGAAAUGGUGCGGUAAGAGCGCACCAGCG-GCGGGGUGACUCG
P.innocua           UGCCGGGGUAAGGGUGAAACGGCGGUGUAAGAGACCACCGGCACCGUGAGCGAUCAC

N.albus             GAGCUAGGUAAACCCCACCCGGAGCAAGACCAGACAGCGUACGACGG----UGUUAG
SM-A26(42)          UGGCAAGGUAAACCCCACUCAGUGCAAAACCAAAUAGGGGAG---AG----------
M.tuberculosis      UGGCUAGGCAAACCCCACCCGAAGCAAGGCCAAGAAGGCCGC---AC----CGAAAG
LGA8                CGGCUCGGCAAACCCCACUCGGAGCAAGACCAAGCAGAGAGGAGCUGCCUGCCACAA
P.innocua           UGGCCAGGUAAACCCCACCCGGAGCAAGACCAGACAGCAGGC---------------

N.albus             ACUGG-----------GAGGGCGGUCCGCCCGAG-----UGCGCGGGUAGGUUGCUA
SM-A26(42)          ------AUG---------GUGCGGCCCGCACU-U-----CUCCCGGGUAGGUUGCUU
M.tuberculosis      GGCCGCGCAGGCGCUUGAGGGUUGCUCGCCCGAG-----CCUGCGGGUAGGCCGCUC
LGA8                GGCGGCACG---------GUUCGGUCCGGACCGUCGUUACUCUCGGGUAGGUCGCUG
P.innocua           ------------------GGGUUGCUCGCCCUUG-----CCUGCGGGUAGGUCGCUG

N.albus             GCCAUCGGCAACGA-UGGUCCGAGAUGGAUGGUCG----------------------
SM-A26(42)          GUAUGUGGUGACAC-AUAUCCCAGAUGAAUGACU-----------------------
M.tuberculosis      GCACCCGGUAACGGUGUGUCC-AGAUGGAUGGUCGCCGCCGUGCCGCCGUUAGCUUG
LGA8                GCGGCGAGCAAUCG-CCGUCCUAGAGAAAUGACCAUC-----GCCGCCC-CAG----
P.innocua           GCCGUCAGCAAUGA-CGGUCCGAGAUGGAUGACC-----------------------

N.albus             ---------------------------------CCGA------CU
SM-A26(42)          ---AUC----CAAGACAGAAC------------CCGGCUU---AC
M.tuberculosis      GGUGGCGGCGCGGAACAGAAUCCGGCUUACAGGCCAACUCGUCCG
LGA8                --GGGCUG-GC---ACAGAAC------------CCGGCUU----A
P.innocua           ---GUC---------------------------CCA-------CG
```

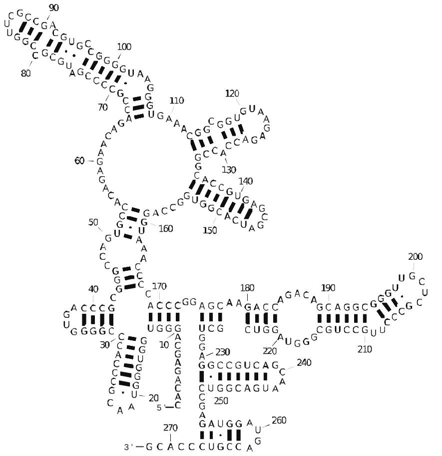
Figure S12. **Predicted alignments by MAFFT for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

```
N.albus         -----------------CACA-GGAC------------------------------
SM-A26(42)      GAGGAAAGUCCGGGCACUAUA-GGGC------------------------------
M.tuberculosis  -----------------CGGAUGAGUUGGCUGGGCGGCCGCGGCUCGCGUAGGGCU
LGA8            GAGGAAAGUCCGGGCUCCACA-GGAC------------------------------
P.innocua       -----------------CACA-GAGC------------------------------

N.albus         ----------------------------------------------------AGGG
SM-A26(42)      ----------------------------------------------------GGAG
M.tuberculosis  UGGAUUCACGAGGUUCAGCGUCGAGUCGAGGAAAGUCCGGACUUCACAGAGCAGGG
LGA8            ----------------------------------------------------AGAG
P.innocua       ----------------------------------------------------AGGG

N.albus         GGCUAACGGCCACCCGGGGUGACCCGCGGGACAGUGCCACAGAGAACAG------A
SM-A26(42)      AGGUAACACCUGGGCGGCGUGAGCCGACGGCAAGUGCAGCAGAGAGAAG------A
M.tuberculosis  UGCUAACGGCAAUCCGAGGUGACUCGCGGGAAAGUGCCACAGAAAACAGACCGCCA
LGA8            CGCUAACGGCGACCGGCCGCAAGGCUCGGGAAAGUGCAACAGAAA-GAC------A
P.innocua       GGGUAACGCCCACCCGGGGUGACCCGCGGGCCAGUGCCACAGAGAACAGACC---G

N.albus         GCCGCUAC----CCU--------CC-A--GGUAGCGGUAAGGGUGAAACGGUGGGG
SM-A26(42)      GCC-UGAC----UC----------------GUC-AGGUAAGGGUGAAAGGGUGCGG
M.tuberculosis  -------------C------------U--CGUGGUGGCAAGGGUGAAACGGUGCGG
LGA8            GCCUCCGCGAGCCCG--------UC-U--CGCGGCGGUAAGGGUGAAAUGGUGCGG
P.innocua       -----GAU----GCGCCGGUUCGCCGACGUGCCGGGGUAAGGGUGAAACGGCGGUG

N.albus         AGCCCACCAGUCCUCGUGGUGACACG-AGGAGCUAGGUAAACCCCACCCGGAGCAA
SM-A26(42)      AGCGCACC-GCGCGAGUGGUAACACUUCGUGGCAAGGUAAACCCCACUCAGUGCAA
M.tuberculosis  AGCGCACCAGCAUUCCGGGUGACCGG-GGUGGCUAGGCAAACCCCACCCGAAGCAA
LGA8            AGCGCACCAGCGG-CGGGGUGACUCG--CCGGCUCGGCAAACCCCACUCGGAGCAA
P.innocua       AGACCACCGGCACCGUGAGCGAUCAC-GGUGGCCAGGUAAACCCCACCCGGAGCAA

N.albus         AGACAGC--GUA-CGACGGUGUUAG---UUCA-------CUGGGAGG-GCGGU---
SM-A26(42)      AAAUAGG--GGAGAGAUGG---------------------------U-GCGGC---
M.tuberculosis  AAGAAGGCCGCACCGAAAGUGCGGC---CGCGCAG-GCGCUU-GAGG-GUUGC---
LGA8            AAGCAGA--GAG-GA-----GCUGCCUGCC-ACAAGG----CGGCGGCACGGUUCG
P.innocua       AGACAGC--AGG-C-------------------------------GG-GUUGC---

N.albus         ----CGCCCGAGUGCGCGGGUAGGUUGCUAGAGGCCAUCGGCAACGA-UGGUCCGA
SM-A26(42)      ----CGCACUUCUCCC-GGGUAGGUUGCUUGAGGUAUGUGGUGACAC-AUAUCCCA
M.tuberculosis  ----CGCCCGAGCCUGCGGGUAGGCCGCUCGAGGCACCCGGUAACGGUGUGU-CCA
LGA8            GGACCGUCGUUACUCUCGGGUAGGUCGCUGGAGGCGGCGAGCAAUCG-CCGUCCUA
P.innocua       ----CGCCCUUGCCUGCGGGUAGGUCGCUGGAGGCCGUCAGCAAUGA-CGGUCCGA

N.albus         GAU--------------------------G----G---------------------
SM-A26(42)      AAU--------------------------GACU-AU------CC------------
M.tuberculosis  GAUGGUCGCCGCCGUGCCGCCGUUAGCUUGGCUGG------------UGGCGGCGC
LGA8            AAU--------------------------GACC-AUCGCCGCCCAGG-----GGC
P.innocua       GAU--------------------------G----A---------------------

N.albus         -------UCG-CCGA--------------CU
SM-A26(42)      ACAGAACCCGGCUU---------------AC
M.tuberculosis  ACAGAAUCCGGCUUACAGGCCAACUCGUCCG
LGA8            ACAGAACCCGGCU----------------UA
P.innocua       -------CCGUCCCA--------------CG
```

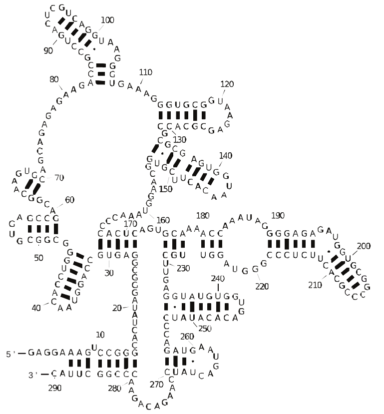
Figure S13. **Predicted alignments by R-Coffee for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**



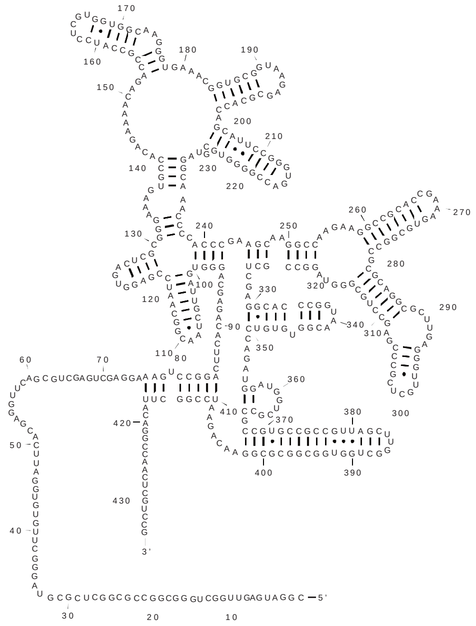
(a) *Nocardioides albus*



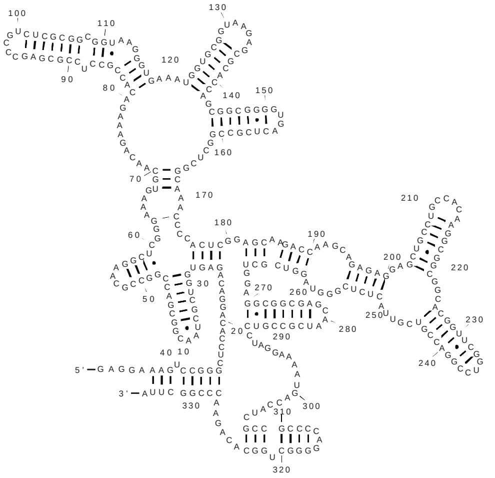
(b) *Propioniferax innocua*



(c) *Salt Marsh A26*



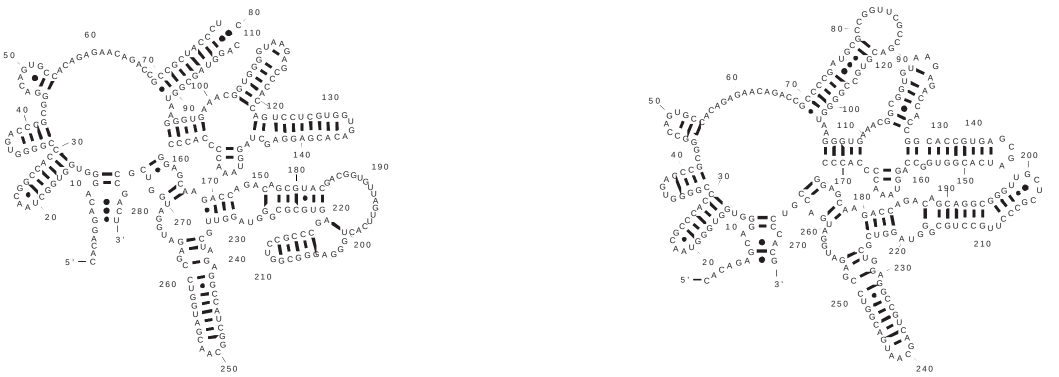
(d) *Mycobacterium tuberculosis*



(e) *Lake Griffy A #8*

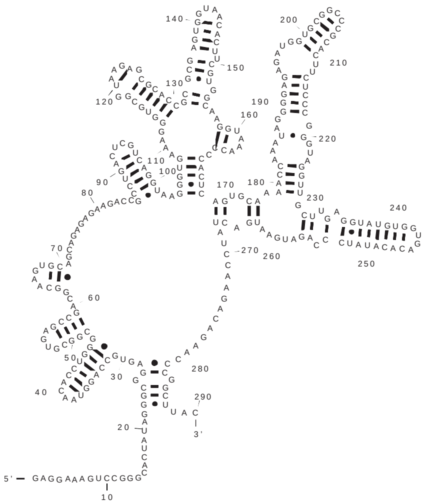
Figure S14. **Predicted structures by TurboFold II for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.** Predicted structures for *Nocardioides albus* (a), *Propioniferax innocua* (b), *Salt Marsh A26* (c), *Mycobacterium*

tuberculosis (d) and *Lake Griffy A #8* (e). 5’ and 3’ ends of sequences are indicated by “5’” and “3’”. A heavy line between two nucleotides indicates the base pairing interaction between the nucleotides.

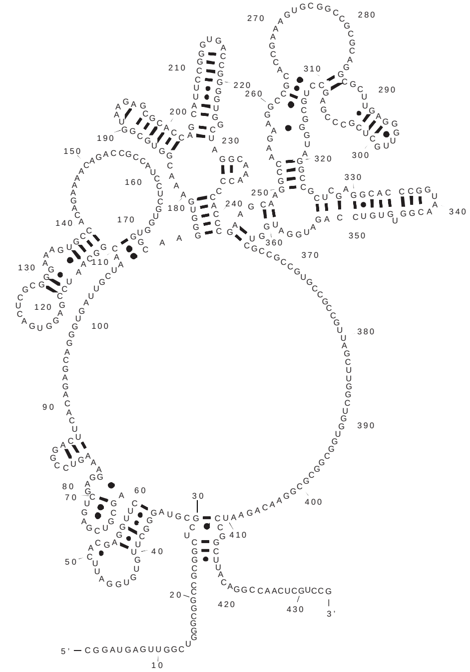


(a) *Nocardioides albus*

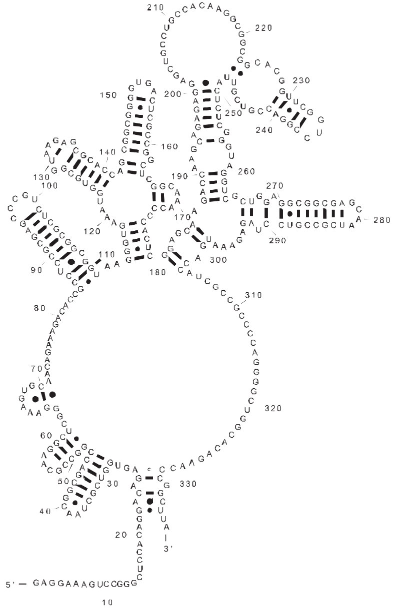
(b) *Propioniferax innocua*



(c) *Salt Marsh A26*



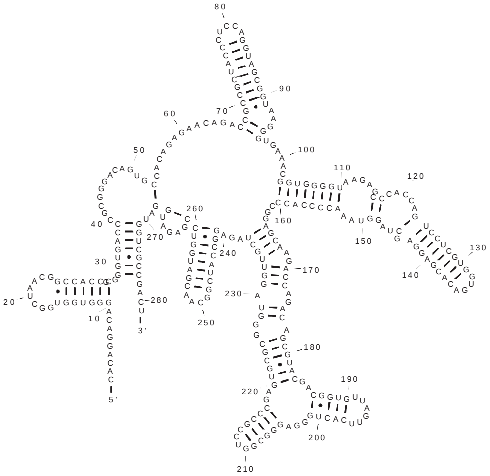
(d) *Mycobacterium tuberculosis*



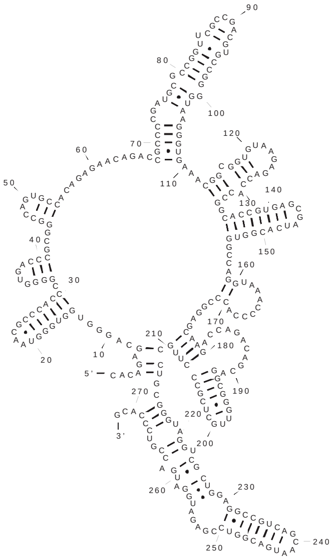
(e) *Lake Griffy A #8*

Figure S15. **Predicted structures by LocARNA for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.** Predicted structures for

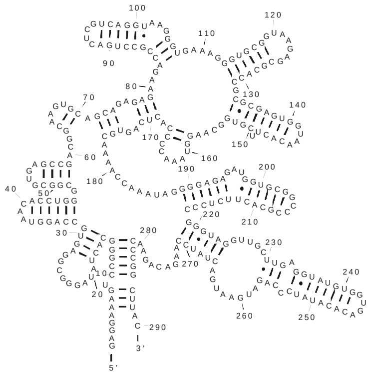
Nocardioides albus (a), *Propioniferax innocua* (b), *Salt Marsh A26* (c), *Mycobacterium tuberculosis* (d) and *Lake Griffy A #8* (e). 5’ and 3’ ends of sequences are indicated by “5’” and “3’”. A heavy line between two nucleotides indicates the base pairing interaction between the nucleotides.



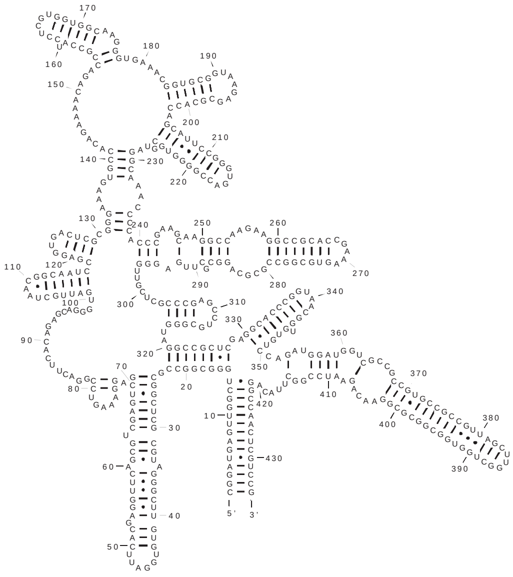
(a) *Nocardioides albus*



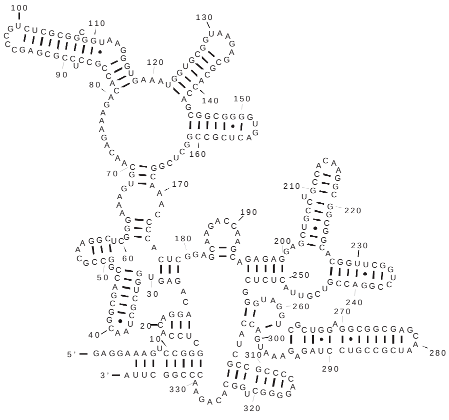
(b) *Propioniferax innocua*



(c) *Salt Marsh A26*



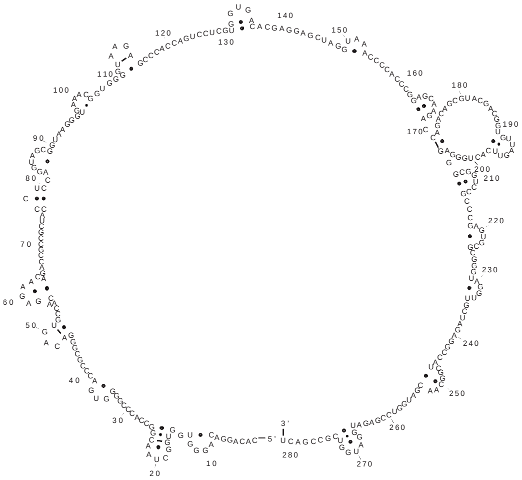
(d) *Mycobacterium tuberculosis*



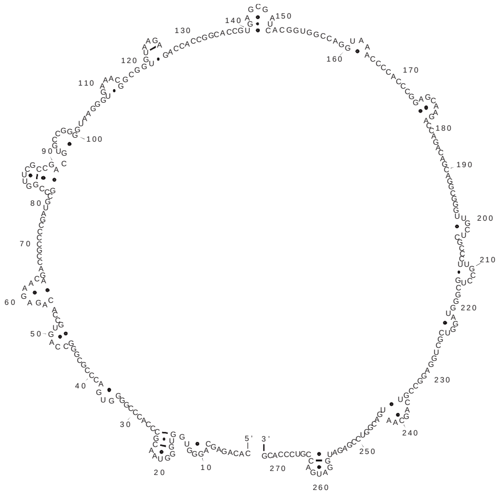
(e) *Lake Griffy A #8*

Figure S16. **Predicted structures by MaxExpect for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.** Predicted structures for

Nocardioides albus (a), *Propioniferax innocua* (b), *Salt Marsh A26* (c), *Mycobacterium tuberculosis* (d) and *Lake Griffy A #8* (e). 5’ and 3’ ends of sequences are indicated by “5’” and “3’”. A heavy line between two nucleotides indicates the base pairing interaction between the nucleotides.

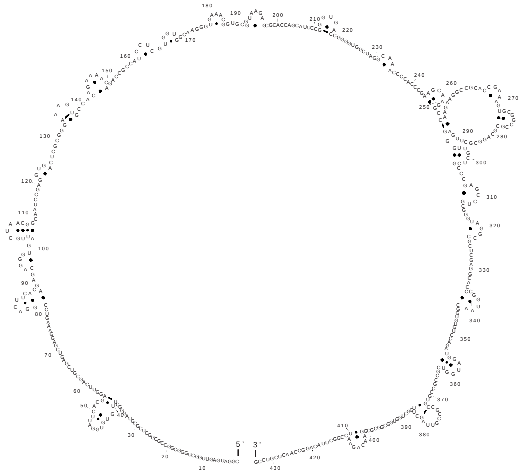


(a) *Nocardioides albus*

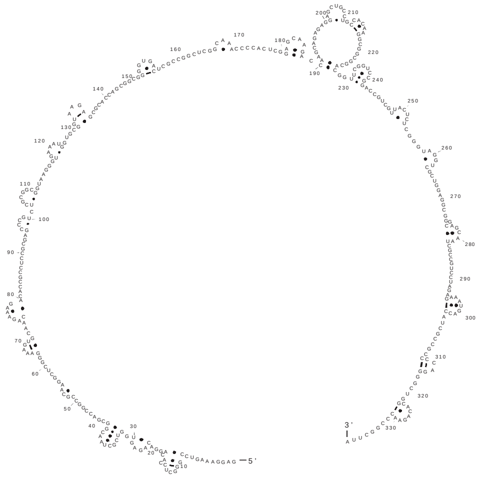


(b) *Propioniferax innocua*

(c) *Salt Marsh A26*



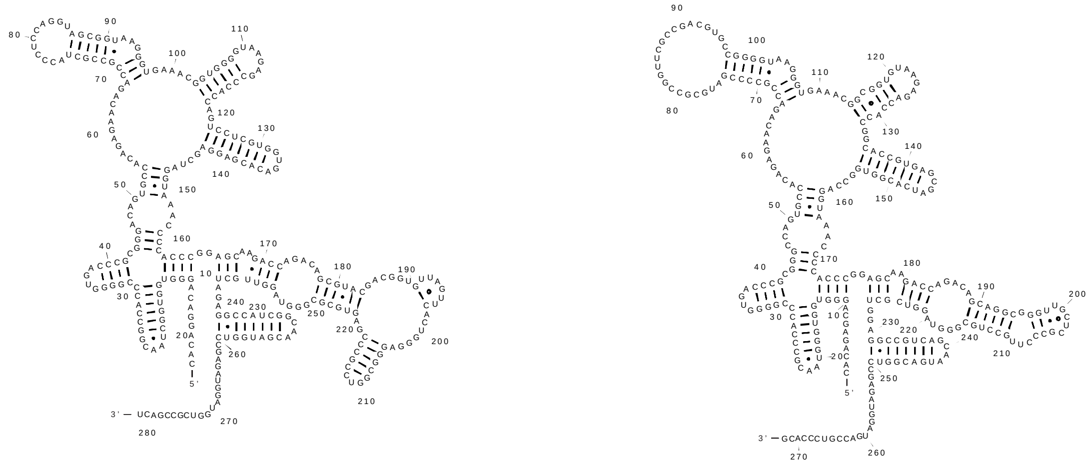
(d) *Mycobacterium tuberculosis*



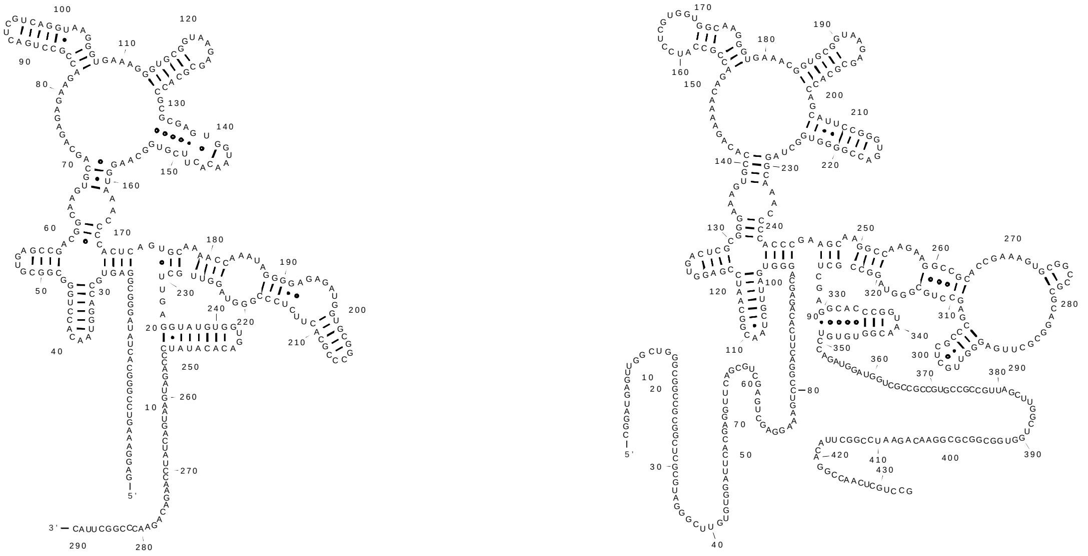
(e) *Lake Griffy A #8*

Figure S17. **Predicted structures by MXSCARNA for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.** Predicted structures for *Nocardioides albus* (a), *Propioniferax innocua* (b), *Salt Marsh A26* (c), *Mycobacterium*

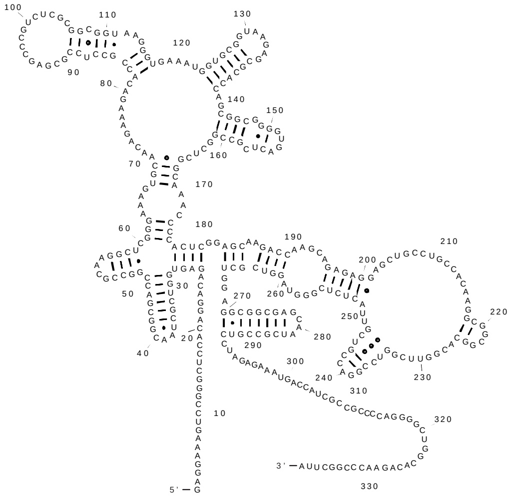
tuberculosis (d) and *Lake Griffy A #8* (e). 5’ and 3’ ends of sequences are indicated by “5’” and “3’”. A heavy line between two nucleotides indicates the base pairing interaction between the nucleotides.



(a) *Nocardioides albus* (b) *Propioniferax innocua*



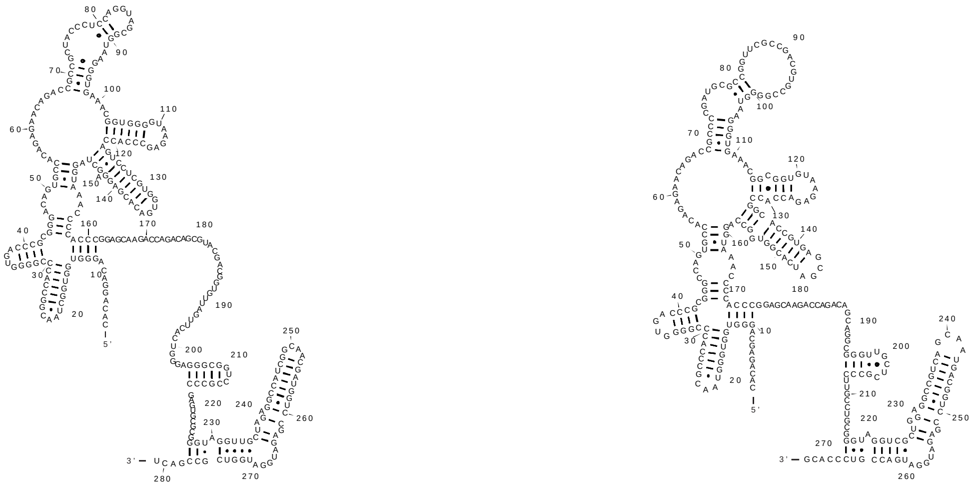
(c) *Salt Marsh A26* (d) *Mycobacterium tuberculosis*



(e) *Lake Griffy A #8*

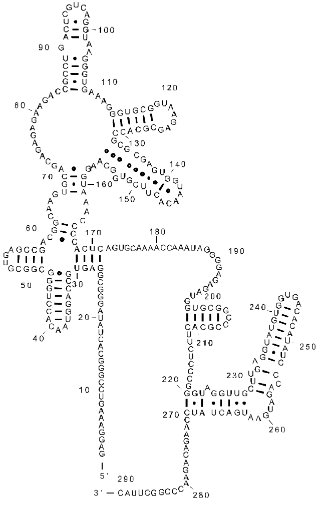
Figure S18. **Predicted structures by RNAalifold (using Clustal Omega) for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

Predicted structures for *Nocardioides albus* (a), *Propioniferax innocua* (b), *Salt Marsh A26* (c), *Mycobacterium tuberculosis* (d) and *Lake Griffy A #8* (e). 5’ and 3’ ends of sequences are indicated by “5’” and “3’”. A heavy line between two nucleotides indicates the base pairing interaction between the nucleotides.

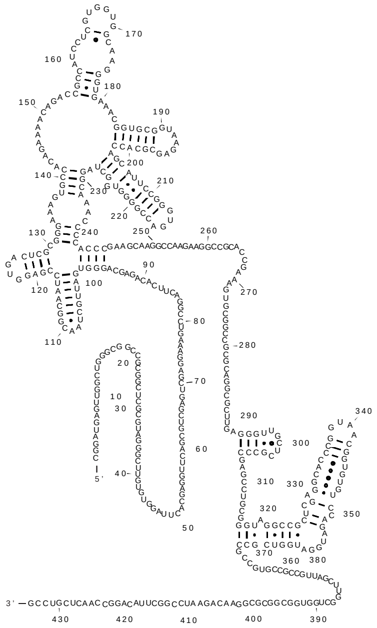


(a) *Nocardioides albus*

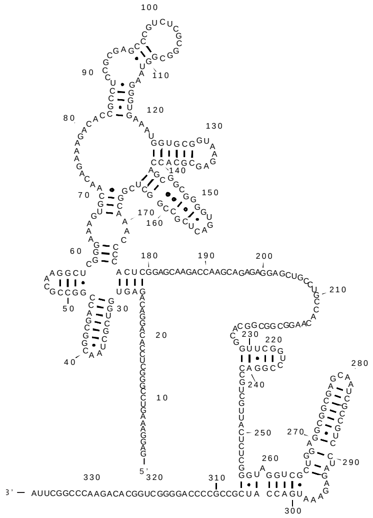
(b) *Propioniferax innocua*



(c) *Salt Marsh A26*



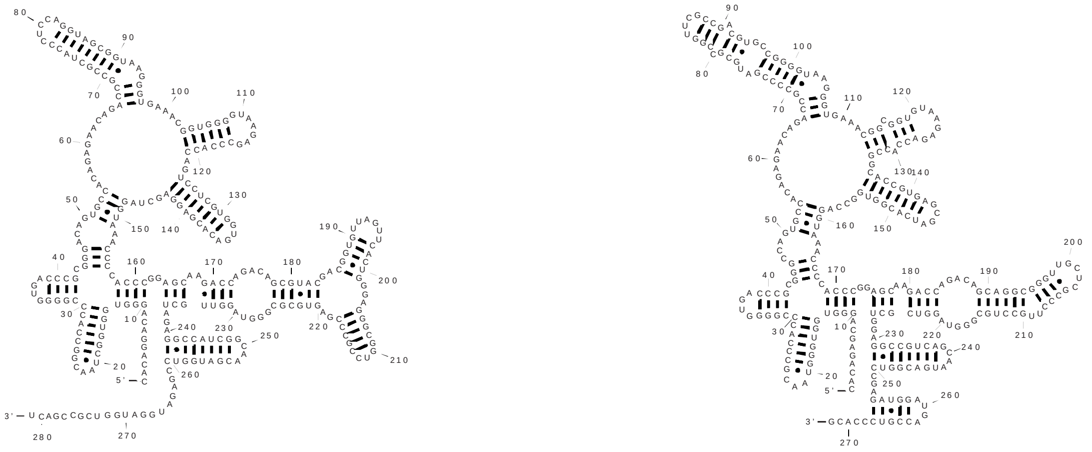
(d) *Mycobacterium tuberculosis*



(e) *Lake Griffy A #8*

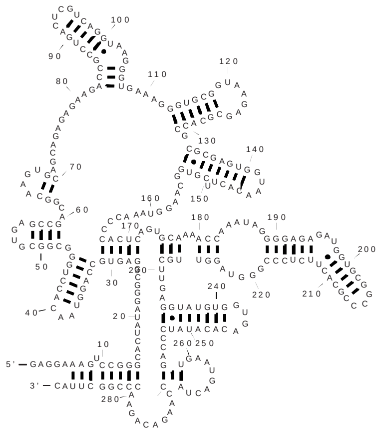
Figure S19. **Predicted structures by RNAalifold (using ClustalW) for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.**

Predicted structures for *Nocardioides albus* (a), *Propioniferax innocua* (b), *Salt Marsh A26* (c), *Mycobacterium tuberculosis* (d) and *Lake Griffy A #8* (e). 5’ and 3’ ends of sequences are indicated by “5’” and “3’”. A heavy line between two nucleotides indicates the base pairing interaction between the nucleotides.

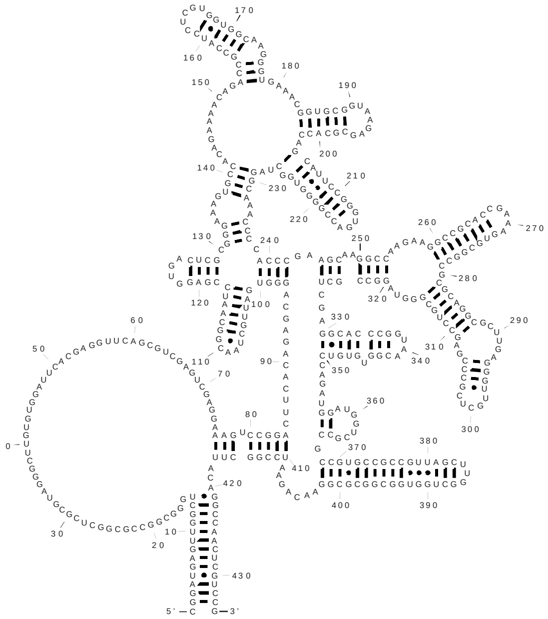


(a) *Nocardioides albus*

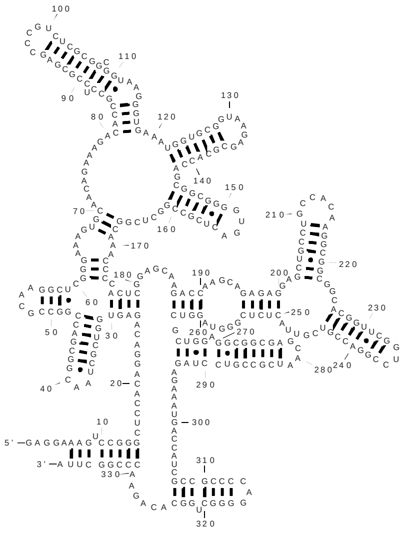
(b) *Propioniferax innocua*



(c) *Salt Marsh A26*



(d) *Mycobacterium tuberculosis*



(e) *Lake Griffy A #8*

Figure S20. **Predicted structures by TurboFold for *Nocardioides albus*, *Propioniferax innocua*, *Salt Marsh A26*, *Mycobacterium tuberculosis* and *Lake Griffy A #8*.** Predicted structures for *Nocardioides albus* (a), *Propioniferax innocua* (b), *Salt Marsh A26* (c), *Mycobacterium*

tuberculosis (d) and *Lake Griffy A #8* (e). 5’ and 3’ ends of sequences are indicated by “5’” and “3’”. A heavy line between two nucleotides indicates the base pairing interaction between the nucleotides.

Section 7. Run time of TurboFold II

Table S3. Run time of the program over the selected families and different number of input sequences. The calculations were run on one core on a machine with an Intel® Core™ i7-4790 CPU @ 3.60GHz.

Family	*H=5 sequences*	*H=10 sequences*	*H=20 sequences*
tRNA	2.57s	7.31s	23.8s
RNase P	55.89s	2m3.67s	9m52.87s
Small subunit RNA	51m3.04s	1h52m30s	7h14m46s

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