Comparative ncRNA Detection in Archaea

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Supplementary Figure 1. Novel putative circRNA from *M. kandleri* (at 1500955-1501112). Alignment and consensus RNA secondary structure with homolog sequences in other archaea; the homologs were identified by blast search at e-value cut-off 0.01 as described in the main text. The figure furthermore reports the genome accession codes of the homolog sequences. The consensus structure and the output figure were generated using RNAalifold [1].

| Species | tRNAscan | | | | cm Search | | |
|--------------------------------|-----------|-----------------|-----------------|-------|-----------|--|--|
| | tRNA Type | Intron Begin l | Intron End | Rank | Bit Score | | |
| Methanopyrus kandleri | Trp | 55,108 | 55,183 | 5. | 21.5 | | |
| Methanopyrus kandleri | Pro | $1,\!499,\!308$ | $1,\!499,\!322$ | 124. | 14.7 | | |
| Methanopyrus kandleri | Pseudo | $1,\!659,\!640$ | $1,\!659,\!691$ | 2. | 25.3 | | |
| Methanopyrus kandleri | Phe | $1,\!639,\!150$ | $1,\!639,\!119$ | not | found | | |
| Methanopyrus kandleri | Cys | 1,062,337 | 1,062,317 | 79. | 15.4 | | |
| Methanopyrus kandleri | Asn | 881,764 | 881,738 | 3. | 25.2 | | |
| Methanopyrus kandleri | Met | 382,127 | 382,092 | 4. | 22.2 | | |
| Sulfolobus solfataricus | Asn | 49,381 | 49,394 | 23. | 12.5 | | |
| Sulfolobus solfataricus | Met | 466,263 | 466,279 | 209. | 9.1 | | |
| Sulfolobus solfataricus | Leu | $637,\!204$ | 637,218 | not | found | | |
| Sulfolobus solfataricus | Leu | $837,\!058$ | 837,073 | 146. | 10.0 | | |
| Sulfolobus solfataricus | Ile | 913,737 | 913,726 | 19. | 12.6 | | |
| Sulfolobus solfataricus | Pro | 898,333 | 898,313 | 242. | 8.9 | | |
| Sulfolobus solfataricus | Thr | 789,727 | 789,713 | 172. | 9.4 | | |
| Sulfolobus solfataricus | Tyr | $642,\!512$ | 642,500 | not | found | | |
| Sulfolobus solfataricus | Ser | $641,\!001$ | 640,978 | 801. | 6.7 | | |
| Sulfolobus solfataricus | Arg | 290,939 | 290,927 | 566. | 7.3 | | |
| Sulfolobus solfataricus | Arg | $249,\!046$ | $249,\!032$ | 219. | 9.0 | | |
| Sulfolobus solfataricus | Thr | $206,\!385$ | 206,373 | 156. | 9.7 | | |
| Sulfolobus solfataricus | Met | $184,\!841$ | $184,\!817$ | 13. | 13.3 | | |
| Sulfolobus solfataricus | Lys | 138,407 | 138,386 | 1420. | 5.7 | | |
| Sulfolobus solfataricus | Lys | $122,\!617$ | 122,595 | 2. | 17.3 | | |
| Sulfolobus solfataricus | Trp | $72,\!831$ | 72,767 | 1321. | 5.9 | | |
| Sulfolobus acidocaldarius | Ser | $512,\!669$ | $512,\!693$ | 62. | 9.6 | | |
| $Sulfolobus \ acidocal darius$ | Leu | $512,\!819$ | $512,\!833$ | 5. | 14.4 | | |
| $Sulfolobus \ acidocal darius$ | Met | $515,\!240$ | 515,257 | 140. | 8.3 | | |
| $Sulfolobus \ acidocal darius$ | Lys | 608,795 | 608,816 | 1. | 16.7 | | |
| $Sulfolobus \ acidocal darius$ | Pro | $1,\!096,\!684$ | 1,096,704 | 145. | 8.3 | | |
| $Sulfolobus \ acidocal darius$ | Met | $1,\!166,\!860$ | 1,166,879 | 10. | 13.4 | | |
| $Sulfolobus \ acidocal darius$ | Asn | $2,\!181,\!266$ | $2,\!181,\!254$ | 129. | 8.5 | | |
| $Sulfolobus \ acidocal darius$ | Gly | $2,\!160,\!121$ | $2,\!160,\!107$ | 988. | 5.5 | | |
| $Sulfolobus \ acidocal darius$ | Arg | $1,\!241,\!011$ | $1,\!240,\!995$ | 4. | 14.8 | | |
| $Sulfolobus \ acidocal darius$ | Thr | $1,\!188,\!440$ | $1,\!188,\!425$ | 73. | 9.4 | | |
| $Sulfolobus \ acidocal darius$ | Leu | $716{,}510$ | 716,493 | 12. | 13.0 | | |
| Sulfolobus acidocaldarius | Cys | $610,\!584$ | 610,569 | not | found | | |
| $Sulfolobus \ acidocal darius$ | Lys | $607,\!184$ | 607,157 | 11. | 13.0 | | |
| $Sulfolobus \ acidocal darius$ | Thr | $563,\!576$ | $563,\!550$ | 8. | 13.9 | | |
| Sulfolobus acidocaldarius | Phe | $458,\!889$ | $458,\!872$ | not | found | | |
| Sulfolobus acidocaldarius | Gly | $458,\!680$ | $458,\!666$ | not | found | | |
| Sulfolobus acidocaldarius | Arg | 138,765 | 138,749 | 9. | 13.7 | | |
| Sulfolobus acidocaldarius | Trp | 49,256 | 49,197 | 21. | 11.4 | | |

Supplementary Table 1. Comparison between tRNA introns accoring to tRNAscan results for *Methanopyrus kandleri*, *Sulfolobus solfataricus*, and *Sulfolobus acidocaldarius* and cm search results.

Supplementary Table 2. Comparison between circularized RNA according to RNAseq read analysis and predicted BHB elements for *Methanopyrus kandleri*. The first two columns give the genomic position of the left and right circularizing bases. "Read Count" gives the number of reads supporting this particular circularization event. For each locus, which was reported to be associated with an BHB element, the Rank in the genomic screen and its bit score is provided. The last column describes the genomic neighborhood. If it is within an annotated gene, its locus tag is given. For loci in intergenic regions the distance to the upstream and downstream gene is given.

| Ι | RNA-seq | | cm Sea | rch | Genomic Surrounding |
|-------------|-------------|------------|----------|-------|--|
| L. junc. | R. junc. | #Count | Rank Bit | Score | ncbi locus tag |
| 69,921 | 69,985 | 2,065 | _ | | $MK0074 \Leftarrow 25nt 77nt \Rightarrow MK0075$ |
| 91,822 | $91,\!904$ | 2,992 | 7,845. | 8.1 | \Leftarrow MK0099 \Rightarrow |
| 205,318 | $205,\!387$ | 12,182 | _ | | $\mathrm{MK0213} \Leftarrow \mathrm{21nt} \ \mathrm{102nt} \Rightarrow \mathrm{MK0214}$ |
| 219,317 | 219,379 | 2,412 | _ | | \Leftarrow MK0233 \Rightarrow |
| 271,148 | 271,216 | 10,072 | 3,546. | 9.8 | \Leftarrow MK0280 \Rightarrow |
| 361,063 | 361,125 | 1,809 | _ | | \Leftarrow MK0371 \Rightarrow |
| 384,798 | $384,\!945$ | 634 | - | | $\mathrm{MK0403} \Leftarrow 665 \mathrm{nt} \ 201 \mathrm{nt} \Rightarrow \mathrm{MK0404}$ |
| 459,461 | $459,\!532$ | 9,830 | 9,039. | 7.7 | \Leftarrow MK0498 \Rightarrow |
| $519,\!288$ | $519,\!358$ | 22,145 | 15,966. | 6.1 | \Leftarrow MK0556 \Rightarrow |
| 520,778 | $520,\!845$ | $13,\!484$ | _ | | $MK0557 \Leftarrow 6nt 269nt \Rightarrow MK0558$ |
| $755,\!163$ | 755,226 | 304 | _ | | \Leftarrow MK0794 \Rightarrow |
| 790,521 | $790,\!585$ | 1,161 | 19,971. | 5.2 | $\mathrm{MK0830} \Leftarrow \mathrm{6nt} \ \mathrm{142nt} \Rightarrow \mathrm{MK0831}$ |
| $993,\!172$ | $993,\!238$ | 50,802 | _ | | \Leftarrow MK1033 \Rightarrow |
| 1,104,263 | 1,104,330 | 6,223 | 17,300. | 5.7 | $\mathrm{MK1128} \Leftarrow 573 \mathrm{nt} \ 260 \mathrm{nt} \Rightarrow \mathrm{MK1129}$ |
| 1,238,104 | 1,238,178 | 9 | 4,630. | 9.2 | $\Leftarrow \mathrm{MK1253} \Rightarrow$ |
| 1,417,298 | 1,417,370 | 9,371 | _ | | $\mathrm{MK1390} \Leftarrow 36\mathrm{nt}\ 224\mathrm{nt} \Rightarrow \mathrm{MK1391}$ |
| 1,417,378 | 1,417,443 | $5,\!658$ | 14,942. | 6.3 | $MK1390 \Leftarrow 116nt \ 144nt \Rightarrow MK1391$ |
| 1,444,847 | 1,444,927 | 2 | _ | | \Leftarrow MK1415 \Rightarrow |
| 1,500,955 | 1,501,112 | $2,\!648$ | - | | $\mathrm{MK1479} \Leftarrow 88\mathrm{nt}\ 205\mathrm{nt} \Rightarrow \mathrm{MK1480}$ |
| 1,506,611 | 1,506,673 | 8,396 | 6,984. | 8.4 | $\Leftarrow \text{MK1486} \Rightarrow$ |

Supplementary Table 3. Circulare RNA in *Sulfolobus solfataricus* [2] are tested for recovery in the cm screen using the consensus model in glocal mode. Additionally, the analysis was redone using the homology loci, if available, in *Sulfolobus acidocaldarius*. The homology search eas conducted with the GotohScan program [3]. The "Start" and "End" columns refer to position in the genomes NC_002754 and NC_007181, respectively.

| | Sulfolobus solfataricus | | | | Sulfolobus acidocaldarius | | | | | |
|--|-------------------------|-----------------|------------|-----|---------------------------|-----------------|-----------------|-----------------------|----------------------|-------|
| | RNA | A-seq | cm | Sea | rch | RNA | -seq | cm | Sea | rch |
| Name | Start | End | Rank | Bit | \mathbf{Score} | Start | End | Rank | Bit | Score |
| 5S rRNA/SSOr02 | 77,945 | 78,067 | 863. | | 6.5 | $1,\!293,\!914$ | $1,\!294,\!035$ | | - | |
| 16S rRNA/SSOr03 | $871,\!658$ | 873,216 | | — | | 1,108,641 | $1,\!107,\!094$ | | _ | |
| 23S rRNA/SSOr04 | $873,\!334$ | $876,\!429$ | | — | | 1,106,947 | $1,\!103,\!875$ | | _ | |
| tRNA-Trp/SSOt04 | 72,767 | 72,831 | $1,\!321.$ | | 5.9 | 49,197 | 49,262 | 548. | | 6.4 |
| tRNA-Lys/SSOt07 | 138,386 | 138,407 | $1,\!420.$ | | 5.7 | 607,138 | 607,204 | 11. | | 13.0 |
| ${\rm tRNA}\text{-}{\rm Met}/{\rm SSOt11}$ | 184,817 | 184,841 | 13. | | 13.3 | - | — | | _ | |
| tRNA-Pro/SSOt42 | 898,313 | 898,333 | 242. | | 8.9 | 1,096,702 | 1,096,684 | 145. | | 8.3 |
| tRNA-Ser/SSOt33 | 640,978 | 641,001 | 1,094. | | 6.2 | 512,691 | $512,\!669$ | 62. | | 9.6 |
| C/D box sR106 | 285,707 | 285,760 | | — | | 2,179,509 | $2,\!179,\!560$ | | _ | |
| C/D box Sso-180 | 362,308 | 362, 369 | | _ | | 669,556 | 669, 612 | | _ | |
| C/D box sR133 | 442,392 | 442,417 | | _ | | _ | — | | _ | |
| C/D box sR102 | 563,241 | 563,296 | | _ | | 1,388,934 | 1,388,984 | | _ | |
| C/D box Sso-sR8 | 647,783 | $647,\!833$ | 601. | | 7.1 | 1,885,917 | 1,885,967 | | _ | |
| C/D box Sso-sR4 | 666, 143 | 666, 186 | 1,779. | | 5.3 | - | - | | _ | |
| C/D box Sso-sR10 | 794, 186 | 794,240 | | _ | | 1,152,443 | 1,152,394 | | _ | |
| C/D box Sso-207 | 816,021 | 816,075 | | _ | | - | - | | _ | |
| C/D box SSOs02 | 829,352 | 829,405 | | _ | | 1,117,732 | $1,\!117,\!685$ | | _ | |
| C/D box Sso-sR12 | 2,189,397 | $2,\!189,\!456$ | | _ | | - | - | | _ | |
| C/D box sR105 | 2,237,915 | 2,237,962 | | _ | | 217,040 | 217,087 | | _ | |
| H/ACA box sR109 | 59,5510 | 595,579 | 308. | | 8.3 | 458,983 | 459,052 | 351. | | 7.0 |
| ncRNA | 442,786 | 442,854 | | _ | | _ | — | | _ | |
| ncRNA | 722,538 | 722,578 | 3005. | | 4.4 | 417,691 | 417729 | | _ | |
| Sso-117 | $1,\!576,\!633$ | $1,\!576,\!671$ | | _ | | _ | — | | _ | |
| Sso-109 | 1,927,228 | $1,\!927,\!258$ | | _ | | _ | — | | _ | |
| 7S rRNA/SSOr01 | 49,977 | 50023 | 839. | | 6.6 | 72,370 | 72,326 | 763. | | 5.9 |
| Sso-214 | $105,\!148$ | 105, 181 | | — | | - | — | | _ | |
| RNase P | 224,732 | 224,765 | | _ | | 586,242 | 586,211 | | _ | |
| Sso-83 | 581,818 | 581,860 | | _ | | _ | — | | _ | |
| ncRNA | $1,\!275,\!500$ | $127,\!5567$ | | _ | | _ | — | | _ | |
| SSO0393 | $343,\!138$ | 343,264 | | _ | | 650,082 | 650,206 | | _ | |
| Intergenic region | $871,\!573$ | 871657 | 1,107. | | 6.2 | 1,108,730 | $1,\!108,\!647$ | | _ | |
| Intergenic region | 873,215 | 873331 | | _ | | 1,107,091 | $1,\!106,\!972$ | | _ | |
| SSO0389 | 335,563 | $335,\!635$ | | _ | | _ | — | | _ | |
| SSO0845 | 725,923 | 726,085 | | _ | | _ | — | | _ | |
| SSO2359 | $2,\!154,\!297$ | $2,\!154,\!322$ | | — | | 782,915 | 782,892 | | _ | |
| SSO2619 | 2,385,872 | $2,\!385,\!901$ | | _ | | 738,654 | $738,\!681$ | | _ | |
| SSO2642 | 2,404,146 | 2,404,146 | | _ | | 2,114,638 | 2,114,694 | | _ | |

Supplementary Table 4. circRNA candidates of *M. kandleri* and *S. acidolarius* with putatively conserved stable secondary structures as predicted by RNAz. As described in the main text, circRNA candidates were identified by mapping RNA-Seq data, homologs were located in all archaeal genomes, potential homologs were aligned, and subsequently evaluated by RNAz. The table lists the candidates that are predicted as putative structural RNAs together with the number of homologous sequences in the locus alignment and the assigned RNAz class probability.

| circRNA ca | andidate le | ocus | Number of | RNAz class |
|------------------|----------------|-----------------------|-----------|-------------|
| Accession | Start | Stop | Seqs | probability |
| NC_003551.1 | $1500955 \ 1$ | 501112 | 10 | 0.9992 |
| $NC_{-}007181.1$ | $1214595 \ 12$ | 214683 | 4 | 0.680306 |
| $NC_{-}007181.1$ | 1254692 1 | 254799 | 4 | 0.938321 |
| NC_007181.1 | $1107137\ 1$ | 107281 | 39 | 0.998730 |
| NC_007181.1 | $1803656 \ 1$ | 803770 | 3 | 0.722219 |
| NC_007181.1 | 183648 | 183733 | 5 | 0.703805 |
| $NC_{-}007181.1$ | $1995955 \ 1$ | 996059 | 3 | 0.619096 |
| NC_007181.1 | 553923 | 554080 | 4 | 0.799874 |
| NC_007181.1 | 753148 | 753230 | 4 | 0.982281 |
| NC_007181.1 | 766362 | 766509 | 4 | 0.646306 |
| $NC_{-}007181.1$ | 773268 | 773364 | 4 | 0.644060 |
| NC_007181.1 | 86425 | 86509 | 3 | 0.554138 |

Supplementary Table 5. Box C/D snoRNA of *M. kandleri* from [4] showing evidence for a BHB element after aligning to the covariance model for box C/D snoRNA sequences based on sequences of *N. equitans* from [5]. Start and stop enclose just the box C/D snoRNA sequences. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences.

| Start | Stop | Strand | Name | transrealign |
|--------|--------|--------|-------------------------|--------------|
| 1815 | 1879 | + | C/D box snoRNA 1 | У |
| 42955 | 43022 | - | C/D box snoRNA 2 | у |
| 44728 | 44789 | + | C/D box snoRNA 3 | У |
| 69897 | 69962 | + | C/D box snoRNA 4 | n |
| 111152 | 111214 | + | C/D box snoRNA 6 | n |
| 137488 | 137553 | + | C/D box snoRNA 7 | у |
| 139707 | 139764 | + | C/D box snoRNA 8 | n |
| 146560 | 146637 | + | C/D box snoRNA 9 | у |
| 147313 | 147372 | + | C/D box snoRNA 10 | n |
| 170072 | 170146 | - | C/D box snoRNA 11 | n |
| 170850 | 170923 | + | C/D box snoRNA 12 | у |
| 172650 | 172747 | - | C/D box snoRNA 13 | n |
| 181467 | 181526 | + | C/D box snoRNA 14 | у |
| 203475 | 203540 | + | C/D box snoRNA 15 | v |
| 205387 | 205458 | - | C/D box snoRNA 17 | v |
| 227948 | 228036 | + | C/D box snoRNA 19 | v |
| 262147 | 262212 | + | C/D box snoRNA 20 | v |
| 271071 | 271136 | - | C/D box snoRNA 21 | v |
| 271151 | 271217 | - | C/D box snoRNA 22 | v |
| 325495 | 325560 | - | C/D box snoRNA 24 | n |
| 325521 | 325579 | - | C/D box snoRNA 25 | v |
| 327758 | 327832 | + | C/D box snoRNA 26 | v |
| 346543 | 346604 | _ | C/D box snoRNA 28 | v |
| 360535 | 360607 | - | C/D box snoRNA 29 | v |
| 383186 | 383259 | + | C'D box snoRNA 31 | n |
| 384878 | 384947 | + | C/D box snoRNA 32 | v |
| 409873 | 409936 | + | C/D box snoRNA 33 | v |
| 459462 | 459533 | - | C/D box snoRNA 34 | n |
| 462530 | 462593 | + | C/D box snoRNA 35 | у |
| 515532 | 515599 | - | C/D box snoRNA 37 | у |
| 518923 | 518988 | - | C/D box snoRNA 38 | у |
| 519204 | 519281 | - | C/D box snoRNA 39 | у |
| 519290 | 519359 | - | C/D box snoRNA 40 | У |
| 520779 | 520847 | + | C/D box snoRNA 41 | У |
| 524051 | 524115 | + | C/D box snoRNA 43 | у |
| 524115 | 524177 | + | C/D box snoRNA 44 | у |
| 524175 | 524238 | + | C/D box snoRNA 45 | У |
| 537577 | 537643 | + | C/D box snoRNA 46 | у |
| 561189 | 561251 | + | C/D box snoRNA 47 | у |
| 582279 | 582357 | + | C/D box snoRNA 48 | У |
| 603202 | 603273 | + | C/D box snoRNA 49 | у |
| 605153 | 605234 | + | C/D box snoRNA 50 | У |
| 627727 | 627790 | - | C/D box snoRNA 52 | у |
| 700325 | 700386 | - | C/D box snoRNA 53 | n |
| 729573 | 729639 | + | C/D box snoRNA 54 | У |
| 751600 | 751664 | + | C/D box snoRNA 55 | У |
| 755168 | 755227 | - | C/D box snoRNA 56 | у |
| 766722 | 766794 | - | $\rm C/D$ box snoRNA 57 | У |
| 772696 | 772766 | + | $\rm C/D$ box snoRNA 58 | У |
| 776072 | 776149 | + | $\rm C/D$ box snoRNA 59 | у |
| 779256 | 779317 | + | C/D box snoRNA 60 | n |
| 779315 | 779376 | - | C/D box snoRNA 61 | n |
| 790520 | 790586 | - | $\rm C/D$ box snoRNA 62 | у |
| 825703 | 825775 | + | $\rm C/D$ box snoRNA 63 | у |
| 830254 | 830325 | + | C/D box snoRNA 64 | у |
| 839773 | 839862 | - | C/D box snoRNA 65 | у |

Supplementary Table 6. Box C/D snoRNA of M. kandleri from [4] showing evidence for a BHB element after aligning to the covariance model for box C/D snoRNA sequences based on sequences of N. equitans from [5]. Start and stop enclose just the box C/D snoRNA sequences. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences.

| Star | t Stop | Strand | Name | transrealign |
|---------|------------------------|--------|--|--------------|
| 84327 | 9 843335 | + | C/D box snoRNA 66 | n |
| 85831 | 3 858382 | + | C/D box snoRNA 67 | v |
| 87975 | 1 879813 | + | C/D box snoRNA 68 | v |
| 87993 | 8 879993 | _ | C/D box snoRNA 69 | v |
| 89248 | 5 892553 | + | C/D box snoBNA 70 | y v |
| 90058 | 5 900653 | + | C/D box snoRNA 71 | J V |
| 91506 | 2 015133 | | C/D box snoRNA 73 | y V |
| 96106 | 3 961126 | L _ | C/D box snoRNA 74 | y V |
| 06311 | 0 063171 | | C/D box shortNA 74 C/D box snoRNA 75 | y V |
| 00217 | 0 002040 | - | C/D box shorting 75 C/D box snoPNA 76 | y |
| 100200 | 0 995242 | | C/D box shortNA 70 | y D |
| 100500 | 11003142 | + | C/D box showing T_{1} | 11 |
| 102200 | 2 1022023 | - | C/D box shortNA 70 | у |
| 102202 | 0 1022090 | - | C/D box shortna 79 | У |
| 102396 | 8 1024031 | + | C/D box snoRNA 80 | У |
| 104367 | 1043750 | - | C/D box snoRNA 81 | n |
| 104810 | 0 1048194 | + | C/D box snoRNA 82 | У |
| 106599 | 2 1066050 | - | C/D box snoRNA 84 | n |
| 106750 | 4 1067564 | - | C/D box snoRNA 85 | n |
| 107336 | 1 1073433 | - | C/D box snoRNA 86 | У |
| 107782 | 5 1077895 | + | C/D box snoRNA 87 | У |
| 109466 | 8 1094726 | - | C/D box snoRNA 88 | n |
| 110427 | 1 1104334 | - | C/D box snoRNA 89 | У |
| 111864 | 0 1118700 | + | C/D box snoRNA 90 | n |
| 116428 | 1 1164371 | + | C/D box snoRNA 92 | n |
| 116526 | 4 1165330 | - | C/D box snoRNA 93 | n |
| 116652 | 9 1166612 | + | C/D box snoRNA 94 | У |
| 117836 | 3 1178424 | - | C/D box snoRNA 95 | n |
| 118241 | 9 1182494 | + | C/D box snoRNA 96 | у |
| 118349 | 0 1183549 | - | C/D box snoRNA 97 | n |
| 118768 | 8 1187763 | + | C/D box snoRNA 98 | n |
| 120476 | 9 1204834 | + | C/D box snoRNA 99 | n |
| 122134 | 9 1221417 | - | C/D box snoRNA 100 | у |
| 123125 | 3 1231340 | + | C/D box snoRNA 101 | n |
| 123169 | 9 1231762 | + | C/D box snoRNA 102 | у |
| 123237 | 1 1232433 | + | C/D box snoRNA 103 | n |
| 123314 | 4 1233210 | + | C/D box snoRNA 104 | v |
| 123341 | 1 1233470 | _ | C/D box snoRNA 105 | v |
| 126341 | 3 1263479 | + | C/D box snoRNA 107 | v |
| 138336 | 4 1383424 | _ | C/D box snoRNA 108 | v |
| 138649 | 8 1386561 | + | C/D box snoRNA 109 | n |
| 141560 | 2 1415673 | _ | C/D box snoRNA 110 | n |
| 141730 | 0 1417370 | - | C/D box snoRNA 111 | v |
| 141737 | 9 1417442 | _ | C/D box snoRNA 112 | v |
| 141744 | 8 1417512 | _ | C/D box spoRNA 113 | J V |
| 1/09// | 8 1/02515 | L _ | C/D box snoRNA 114 | y V |
| 140319 | 5 1403102 | | C/D box snoRNA 115 | J V |
| 140522 | 0 1405300 | | C/D box spoRNA 116 | y V |
| 150661 | 1 1506674 | | C/D hov snoPNA 117 | y v |
| 150979 | 3 1508700 | - | C/D box shorting 117 | y V |
| 151040 | 01510576 | - | C/D box shorting 110 | y r |
| 1591049 | 5 1591140 | _ | C/D box showing 119 | 11 |
| 156100 | 0 1561004 | | C/D box showing 120 | y r |
| 150123 | 4 1001294 6 1507011 | + | C/D box shorting 121 | 11 |
| 108/14 | | - | C/D box short NA 122 | У |
| 160599 | | + | C/D box snoking 123 | n |
| 160721 | 91007287 | - | U/D DOX SNORNA 124 | У |
| 1163373 | 3 1633798 | + | U/D box snoRNA 126 | У |

Supplementary Table 7. Box C/D snoRNA of M. kandleri from [4] where no BHB element could be found after aligning to the covariance model for box C/D snoRNA sequences based on sequences of N. equitans from [5]. Start and stop enclose just the box C/D snoRNA sequences. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences.

| Start | Stop | Strand | Name | transrealign |
|---------|---------|--------|-------------------|--------------|
| 91821 | 91905 | + | C/D box snoRNA5 | у |
| 205310 | 205380 | - | C/D box snoRNA16 | n |
| 219316 | 219380 | + | C/D box snoRNA18 | n |
| 274089 | 274184 | + | C/D box snoRNA23 | у |
| 333110 | 333179 | + | C/D box snoRNA27 | У |
| 361062 | 361156 | - | C/D box snoRNA30 | n |
| 509783 | 509880 | + | C/D box snoRNA36 | у |
| 520852 | 520933 | + | C/D box snoRNA42 | у |
| 605300 | 605373 | + | C/D box snoRNA51 | У |
| 902800 | 902861 | - | C/D box snoRNA72 | n |
| 1055548 | 1055621 | + | C/D box snoRNA83 | n |
| 1157340 | 1157415 | + | C/D box snoRNA91 | у |
| 1234309 | 1234398 | - | C/D box snoRNA106 | n |
| 1607250 | 1607305 | + | C/D box snoRNA125 | У |

Supplementary Table 8. Box C/D snoRNA sequences of *N. equitans* from [5] used to build a the covariance model for cetection of box C/D snoRNA with a BHB element. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences. Sequences with (*) couldn't be predicted in [5] such that now name was given. We kept the order of sequences and so the name consists of a number in between.

| Start | Stop | Strand | Name | transrealion |
|--------|--------|--------|--------------------|--------------|
| Duare | Dtop | Strand | | transreangn |
| 46922 | 46979 | - | C/D box snoRNA1b* | У |
| 54028 | 54085 | - | C/D box snoRNA2 | n |
| 90898 | 90954 | - | C/D box snoRNA3 | У |
| 113577 | 113628 | - | C/D box snoRNA4 | n |
| 144953 | 145004 | - | C/D box snoRNA5 | У |
| 145962 | 146019 | - | C/D box snoRNA6 | у |
| 164113 | 164168 | - | C/D box snoRNA7 | у |
| 248187 | 248239 | - | C/D box snoRNA7b* | n |
| 282915 | 282973 | - | C/D box snoRNA8 | у |
| 315990 | 316045 | - | C/D box snoRNA10 | у |
| 323041 | 323094 | - | C/D box snoRNA11b* | n |
| 328897 | 328949 | - | C/D box snoRNA13 | у |
| 337448 | 337505 | - | C/D box snoRNA15 | у |
| 371860 | 371917 | - | C/D box snoRNA16 | у |
| 375354 | 375406 | - | C/D box snoRNA17 | y |

Supplementary Table 9. Box C/D snoRNA sequences of *N. equitans* from [5] where a BHB motif could be detected. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences. Sequences with (*) couldn't be predicted in [5] such that now name was given. We kept the order of sequences and so the name consists of a number in between.

| Start | Stop | Strand | Name | transrealign |
|--------|--------|--------|--------------------|--------------|
| 45779 | 45831 | + | C/D box snoRNA1 | n |
| 297634 | 297687 | + | C/D box snoRNA9 | n |
| 318915 | 318968 | + | C/D box snoRNA11 | у |
| 334552 | 334611 | + | C/D box snoRNA14 | у |
| 359412 | 359470 | + | C/D box snoRNA15a* | у |
| 362755 | 362808 | + | C/D box snoRNA15b* | у |
| 382458 | 382509 | + | C/D box snoRNA18 | у |
| 401223 | 401280 | + | C/D box snoRNA18b* | у |
| 403464 | 403522 | + | C/D box snoRNA19 | n |

Supplementary Table 10. Box C/D snoRNA sequences of N. equitans from [5] where no clear BHB motif could be detected. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences. Sequences with (*) couldn't be predicted in [5] such that now name was given. We kept the order of sequences and so the name consists of a number in between.

| Start | Stop | Strand | Name | transrealign |
|--------|--------|--------|--------------------|--------------|
| 325371 | 325424 | + | C/D box snoRNA12 | n |
| 384218 | 384278 | + | C/D box snoRNA18a* | у |

Supplementary Table 11. Box C/D snoRNA sequences of S. solfataricus from [6, 7] where a BHB motif could be detected. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences. Sequences with (*) couldn't be predicted in [5] such that now name was given. We kept the order of sequences and so the name consists of a number in between.

| Start | Stop | Strand | Name | $\operatorname{transrealign}$ |
|---------|---------|--------|-----------------------|-------------------------------|
| 362293 | 362384 | + | C/D box snoRNA 180 | у |
| 794171 | 794255 | + | C/D box snoRNA 10 | у |
| 829337 | 829420 | + | C/D box snoRNA 02 | у |
| 2189382 | 2189471 | + | C/D box snoRNA 12 | n |
| 1274759 | 1274879 | - | C/D box snoRNA $n2^*$ | n |
| 2003526 | 2003688 | + | C/D box snoRNA 67 | n |
| 2111320 | 2111468 | - | C/D box snoRNA 67b* | n |

Supplementary Table 12. Box C/D snoRNA sequences of *S. solfataricus* from [2, 7] where no clear BHB motif could be detected. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences. Sequences with (*) couldn't be predicted in [5] such that now name was given. We kept the order of sequences and so the name consists of a number in between.

| Start | Stop | Strand | Name | $\operatorname{transrealign}$ |
|---------|---------|--------|--------------------|-------------------------------|
| 285692 | 285775 | + | C/D box snoRNA 106 | n |
| 442377 | 442432 | + | C/D box snoRNA 133 | n |
| 563226 | 563311 | + | C/D box snoRNA 102 | у |
| 816006 | 816090 | + | C/D box snoRNA 207 | у |
| 2237900 | 2237977 | + | C/D box snoRNA 105 | у |
| 22518 | 22604 | + | C/D box snoRNA 195 | n |
| 163939 | 164015 | + | C/D box snoRNA 65 | n |
| 442348 | 442449 | + | C/D box snoRNA n1* | n |
| 590308 | 590410 | - | C/D box snoRNA 101 | n |
| 837791 | 837886 | - | C/D box snoRNA 125 | n |
| 885646 | 885840 | - | C/D box snoRNA 110 | у |
| 1401961 | 1402198 | - | C/D box snoRNA n3* | у |
| 2369460 | 2369600 | + | C/D box snoRNA 93 | n |

Supplementary Table 13. Box C/D snoRNA sequences of *S. acidocaldarius* from [6] [2] where a BHB motif could be detected. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences.

| Start | Stop | Strand | Name | transrealign |
|---------|---------|--------|--------------------------|--------------|
| 2179492 | 2179578 | + | C/D box snoRNA 18 | n |
| 793941 | 794027 | - | C/D box snoRNA 17 | у |
| 2196878 | 2196963 | + | C/D box snoRNA 16 | n |
| 1400376 | 1400445 | + | C/D box snoRNA 15 | У |
| 1117434 | 1117521 | - | C/D box snoRNA 14 | у |
| 345672 | 345759 | + | C/D box snoRNA 13 | у |
| 86078 | 86167 | + | C/D box snoRNA 12 | n |
| 131771 | 131859 | + | C/D box snoRNA 11 | У |
| 217020 | 217107 | + | C/D box snoRNA 10 | У |
| 42115 | 42207 | + | C/D box snoRNA 9 | у |
| 2175730 | 2175814 | - | C/D box snoRNA 8 | у |
| 393832 | 393923 | - | C/D box snoRNA 7 | У |
| 368172 | 368254 | + | C/D box snoRNA 6 | у |
| 449443 | 449530 | - | C/D box snoRNA 5 | у |
| 2217096 | 2217181 | - | C/D box snoRNA 4 | n |
| 1117670 | 1117755 | + | C/D box snoRNA 3 | у |
| 1075489 | 1075575 | + | C/D box snoRNA 2 | у |
| 1220543 | 1220628 | + | C/D box snoRNA 1 | n |
| 2179494 | 2179575 | + | $\rm C/D$ box snoRNA 106 | n |
| 669541 | 669627 | + | $\rm C/D$ box snoRNA 102 | У |
| 1388919 | 1388999 | + | C/D box snoRNA 180 | У |
| 217025 | 217102 | + | C/D box snoRNA 105 | У |

Supplementary Table 14. Box C/D snoRNA sequences of *S. acidocaldarius* from [6] [2] where no clear BHB motif could be detected. For the alignment, 15 nucleotides of flanking sequences were added on both sides of the box C/D snoRNA sequences.

| Start | Stop | Strand | Name | transrealign |
|---------|---------|--------|-------------------|--------------|
| 1152379 | 1152458 | - | C/D box snoRNA 10 | У |
| 1117670 | 1117747 | - | C/D box snoRNA 02 | У |

Supplementary Table 15. Details of the RNASeq analysis. Sequences were mapped to the reference genome with segemehl [8, 9] and remapped with lack, another program of the segemehl suite as well as transrealign which was used to extract the split reads. As *N. equitans* and *I. hospitalis* live in a parasymbiontic manner, the RNASeq data(*) (in total 16020851 reads) was mapped together to both reference genomes at the same time and splitted afterwards.

| Species | pooled reads | mapped reads | remapped reads | split reads |
|-------------------|--------------|--------------|----------------|-------------|
| M. kandleri | 19242863 | 13736113 | 2153772 | 128603 |
| S. acidocaldarius | 26023157 | 22762924 | 360912 | 108283 |
| S. solfataricus | 8976013 | 7764310 | 878127 | 74143 |
| N. equitans | * | 10728929 | - | 25721 |
| I. hospitalis | * | 5567812 | - | 42757 |

| MCAI | MCA9 |
|-----------------------|-------------------------|
| | |
| Start Stop Strand | Start Stop Strand |
| 143515 143632 + | 31858 31995 + |
| $163201 \ 163467 \ +$ | 40439 40539 + |
| 325320 325593 + | 262091 262244 + |
| 351364 351601 + | 325412 325593 + |
| 399364 399622 + | 384725 384960 + |
| 490225 490354 + | 497434 497516 + |
| 518084 518344 + | 507442 507586 + |
| 518229 518322 + | 509798 509895 + |
| 518263 518344 + | 510730 510856 + |
| 522017 522129 + | 517768 517974 + |
| 585393 585475 + | 517864 518003 + |
| 585629 585772 + | 520831 520932 + |
| 585946 586138 + | 576556 576720 + |
| 586198 586414 + | 582234 582370 + |
| 586751 586957 + | 585979 586110 + |
| 586869 587025 + | 588030 588271 + |
| 587307 587436 + | 693682 693927 + |
| 587746 587871 + | 739727 739822 + |
| 673889 673968 + | 744880 745039 + |
| $1019844 \ 1020094 +$ | 779240 779332 + |
| $1022388 \ 1022636 +$ | 879679 879914 + |
| $1112401 \ 1112677 +$ | $1183460 \ 1183557 +$ |
| $1221433\ 1221523$ + | $1219327 \ 1219497 +$ |
| 1243818 1244084 + | $1232353 \ 1232448 +$ |
| $1343553\ 1343808 +$ | $1252557 \ 1252674 +$ |
| | $1259272 \ 1259422 +$ |
| | $1279749 \ 1279961 +$ |
| | $1415483 \ 1415647 +$ |
| | $1540620 \ 1540755 \ +$ |
| | $1688642 \ 1688778 +$ |

Supplementary Table 16. New splicesites with BHB elements found in *M. kandleri* with MSA1 and MSA2. Start and Stop positions include the 15nt flanking sequence.

Supplementary Table 17. New splicesites with BHB elements found in *S. acido-caldarius* with MSA1 and MSA2. Start and Stop positions include the 15nt flanking sequence.

| | MSA1 | | | MSA2 | |
|---------|---------|--------|---------|---------|--------|
| Start | Stop | Strand | Start | Stop | Strand |
| 49182 | 49271 | + | 49182 | 49271 | + |
| 72311 | 72447 | + | 73220 | 73336 | + |
| 708452 | 708600 | + | 97306 | 97393 | + |
| 789407 | 789634 | + | 267193 | 267304 | + |
| 848243 | 848477 | + | 450894 | 450975 | + |
| 1013752 | 1013976 | + | 677737 | 677855 | + |
| 1029123 | 1029366 | + | 624809 | 624949 | + |
| 1030222 | 1030393 | + | 639014 | 639153 | + |
| 1270855 | 1270973 | + | 753133 | 753241 | + |
| 1285009 | 1285134 | + | 808261 | 808346 | + |
| 1366353 | 1366466 | + | 913155 | 913261 | + |
| 1785213 | 1785353 | + | 986137 | 986328 | + |
| | | | 1126119 | 1126204 | + |
| | | | 1212710 | 1212788 | + |
| | | | 1327997 | 1328130 | + |
| | | | 1381538 | 1381622 | + |
| | | | 1648994 | 1649075 | + |
| | | | 2072492 | 2072665 | + |

Supplementary Table 18. New splicesites with BHB elements found in *N. equitans* with MSA1 and MSA2. Start and Stop positions include the 15nt flanking sequence.

| MSA1 | MSA2 |
|---------------------|---------------------|
| Start Stop Strand | Start Stop Strand |
| $396266\ 396385 +$ | $403449 \ 403535 +$ |
| $433125\ 433234$ + | |
| $396539 \ 396812 +$ | |

Supplementary Table 19. New splicesites with BHB elements found in *I. hospitalis* with MSA1 and MSA2. Start and Stop positions include the 15nt flanking sequence.

| MSA1 | MSA2 |
|-----------------------|-----------------------|
| Start Stop Strand | Start Stop Strand |
| $603277\ 603363$ + | 3921 4027 + |
| $733481 \ 733720 \ +$ | 281959 282165 + |
| $798681 \ 798876 +$ | 602934 603198 + |
| | 731896 732076 + |
| | 733248 733446 + |
| | 945608 945702 + |
| | $1115636 \ 1115739 +$ |

Supplementary Table 20. New splicesites with BHB elements found in *S. solfa-taricus* with MSA1 and MSA2. Start and Stop positions include the 15nt flanking sequence.

| MSA1 | MSA2 |
|-------------------|-----------------------|
| Start Stop Strand | Start Stop Strand |
| | 763512 763600 + |
| | 872878 873132 + |
| | 872918 873156 + |
| | 875970 876218 + |
| | 876228 876426 + |
| | $2361384\ 2361469 +$ |
| | $2939076 \ 2939162 +$ |

References

- Bernhart, S.H., Hofacker, I.L., Will, S., Gruber, A.R., Stadler, P.F.: RNAalifold: improved consensus structure prediction for RNA alignments. BMC Bioinformatics 9 (2008) 474
- Danan, M., Schwartz, S., Edelheit, S., Sorek, R.: Transcriptome-wide discovery of circular RNAs in archaea. Nucleic Acids Res. 40 (2012) 3131–3142
- Hertel, J., de Jong, D., Marz, M., Rose, D., Tafer, H., Tanzer, A., Schierwater, B., Stadler, P.F.: Non-coding RNA annotation of the genome of *Trichoplax adhaerens*. Nucleic Acids Res. **37** (2009) 1602–1615
- 4. Su, A.A.H., Tripp, V., Randau, L.: RNA-Seq analyses reveal the order of tRNA processing events and the maturation of C/D box and CRISPR RNAs in the hyperthermophile Methanopyrus kandleri. Nucleic Acids Res. 41 (2013) 6250–6258
- Randau, L.: RNA processing in the minimal organism Nanoarchaeum equitans. Genome Biology 13 (2012)
- Omer, A.D., Lowe, T.M., Russell, A.G., Ebhardt, H., Eddy, S.R., Dennis, P.P.: Homologs of small nucleolar rnas in archaea. Science 288(5465) (2000) 517–522
- Wurtzel, O., Sapra, R., Chen, F., Zhu, Y., Simmons, B.A., Sorek, R.: A single-base resolution map of an archaeal transcriptome. Genome Res. 20 (2010) 133–141
- Hoffmann, S., Otto, C., Kurtz, S., Sharma, C.M., Khaitovich, P., Vogel, J., Stadler, P.F., Hackermüller, J.: Fast mapping of short sequences with mismatches, insertions and deletions using index structures. PLoS Comp. Biol. 5 (2009) e1000502
- Hoffmann, S., Otto, C., Doose, G., Tanzer, A., Langenberger, D., Christ, S., Kunz, M., Holdt, L.M., Teupser, D., Hackermüller, J., Stadler, P.F.: A multi-split mapping algorithm for circular RNA, splicing, trans-splicing, and fusion detection. Genome Biology 15 (2014) R34