

A

16S-U934
 Pae-sR201
 Par-sR201
 Pca-sR201
 Pis-sR201
 Tneu-sR201
 Pog-sR201

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  --- g g c c g c a a --- c Ψ a a g g u u
  g c c c c u g g c g u - g g g g c g u c g a g g c g c c a c g g c C G C U G U G A A A A C G C C C C C U C C A A G G G G C G C A C A
  g c c c c u g g c g u - g g g g u g u c g a g u c g c c g u g u g g g c g c u g u g a a a a c g c c c c c u c c g c g g g g c g u a c a
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B

23S-U2023
 Pae-sR204
 Par-sR204
 Pca-sR204
 Pis-sR204
 Tneu-sR204
 Pog-sR204

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  - g u c u c a - - - - - a Ψ g g g g c c c g g
  - - g a g u g c c g c c g a g a g c c G C G C - G C G C G G C C C A U G A G C G G C G G U U C C C C G G C C - - A U A
  - - G A G U G C C G C C C G A G A G C C G C U - U C U G C G G C C C A G U G A C C G G C G G U U C C C C G G C U - - A U U
  - - G A G U G C C G C C C G A G U G C C G C A G A U G U C G G G C C G A U G A A A G G C G G U U C C C C G G C U - - U U U
  A U A G A G U G C C G C U G A G U G C C G C C - U A G G C G G C U C U A U G A U A G U C G G C C C C C G G C C U U A A A
  - - g a g u g c c g c c g a g c g c c g c c u - u u g g c g g c c c c g u g a a g u c g g c c c c g g c u u - a a a
  - - g a g u g c c g c c c g a g a g c c g c c u - u c u g c g g c c - c a g u g a u c g g c g g u u c c c c g g c u - - a u u
  
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C

23S-U2029
 Pae-sR205
 Par-sR205
 Pca-sR205
 Pis-sR205
 Tneu-sR205
 Pog-sR205

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  --- - - - - g Ψ c u c a a u g g g g c c c g g
  U U - - - - A G C C C U A G G U A A C A C C C C U G A G C - G G G G C G G A U G A G C C U G G C G G G U U A C C C C - G G C C A A A
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  - - - - - g c c c u a g g c a a c a c c c c u g a g - g g g g c g u a u g a g c c u g g c g g g u u a c c c c - g g c c a u a
  - - - - - a c c c u g g g c a a c a c c c c u g a g g g g g c g a a u g a g c c u g g u g g g u u a c c c c - g g c c a u a
  
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D

23S-U1978
 Pae-sR208
 Par-sR208
 Pca-sR208
 Pis-sR208
 Pog-sR208
 Tneu-sR208

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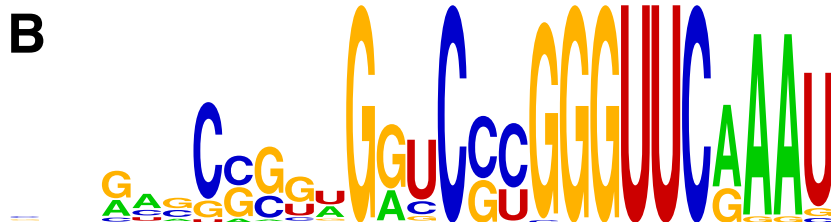
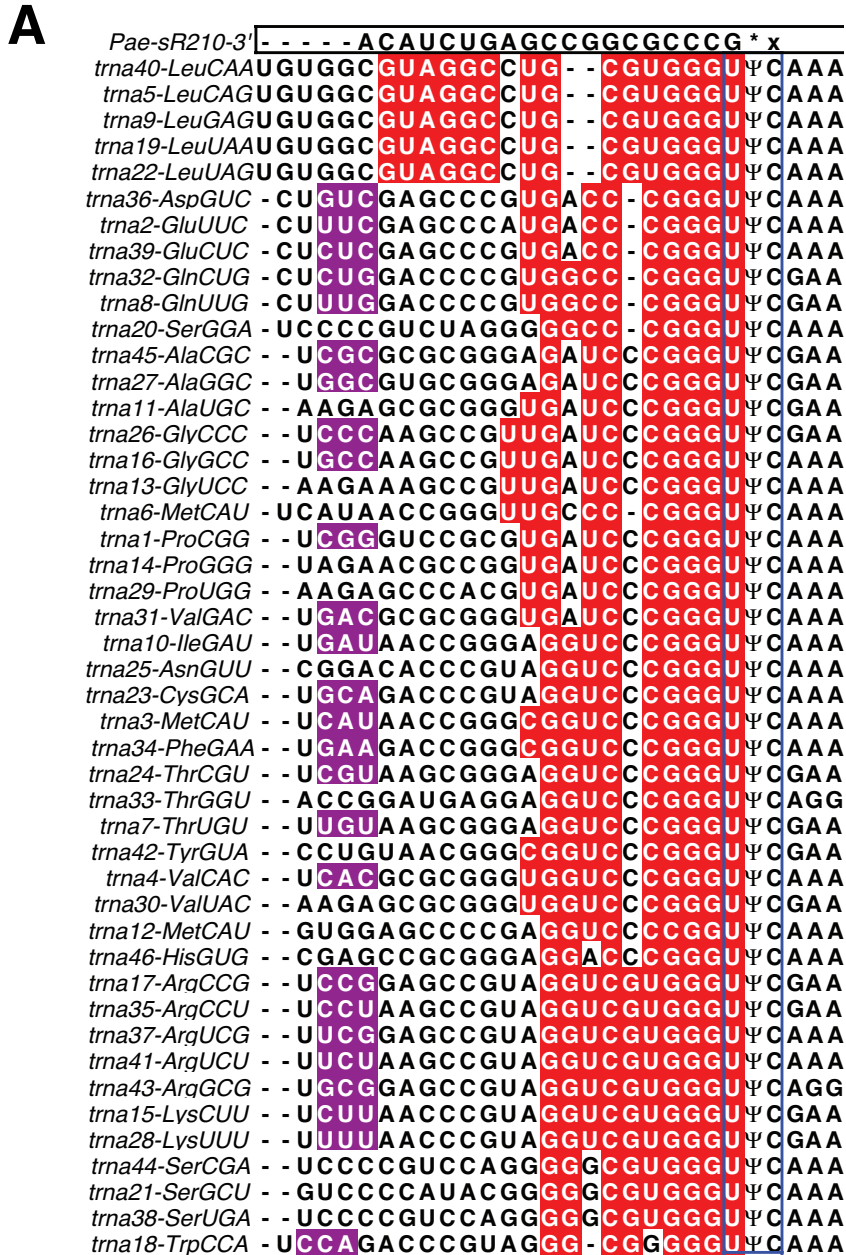
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16S-U8
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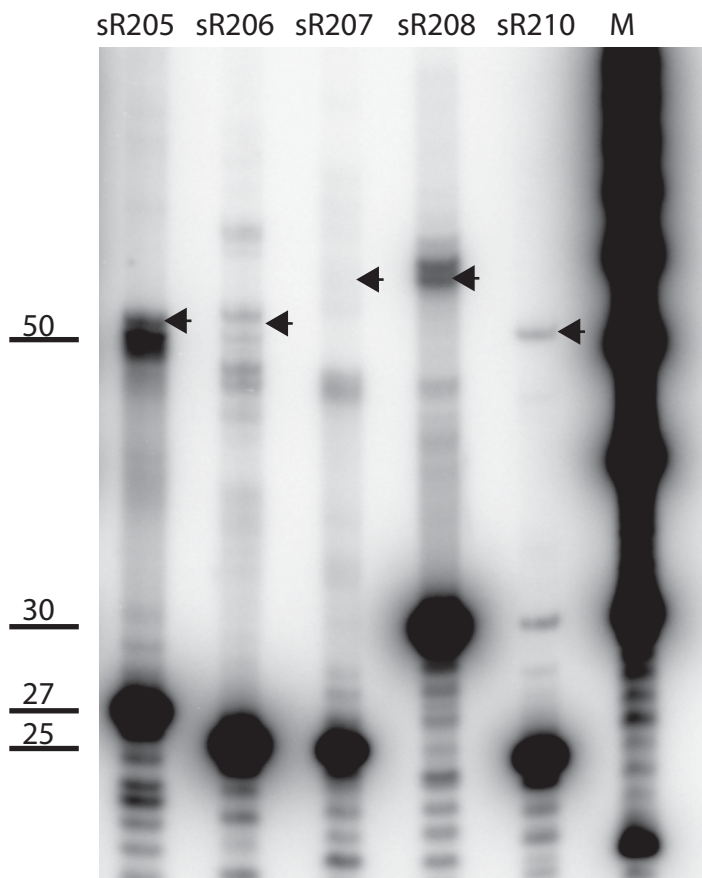
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  g g c a g g a u c g g c c u c g a g u g g g c c g g c - - g g c c c c u a u g a g a g g g c c c g g a u c u a a g c g a a a a
  
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Supplemental Figure 1: Additional guide alignments from each of the sequenced *Pyrobaculum*. sR201 (panel A), sR204 (B), sR205 (C), sR208 (D) and sR209 (E) are shown. Target RNA sequence shown in top sequence row of each panel. Predicted Watson-Crick and G-U base-pairing between target and sRNA guide regions shown (red). Stem pairing of the lower stem (aqua), intermediate stem (dark blue) and upper apical stem (purple) are indicated. Lower stem/guide base-pairing is uncertain for sR201 (Panel A) in region where target sequence (red) overlaps lower stem (aqua). The kink-turn motif is indicated in orange. RNA sequences are shown in upper case for sequences recovered in RNA sequencing, lower case when sequence is inferred by conservation of aligned features in genome sequence.

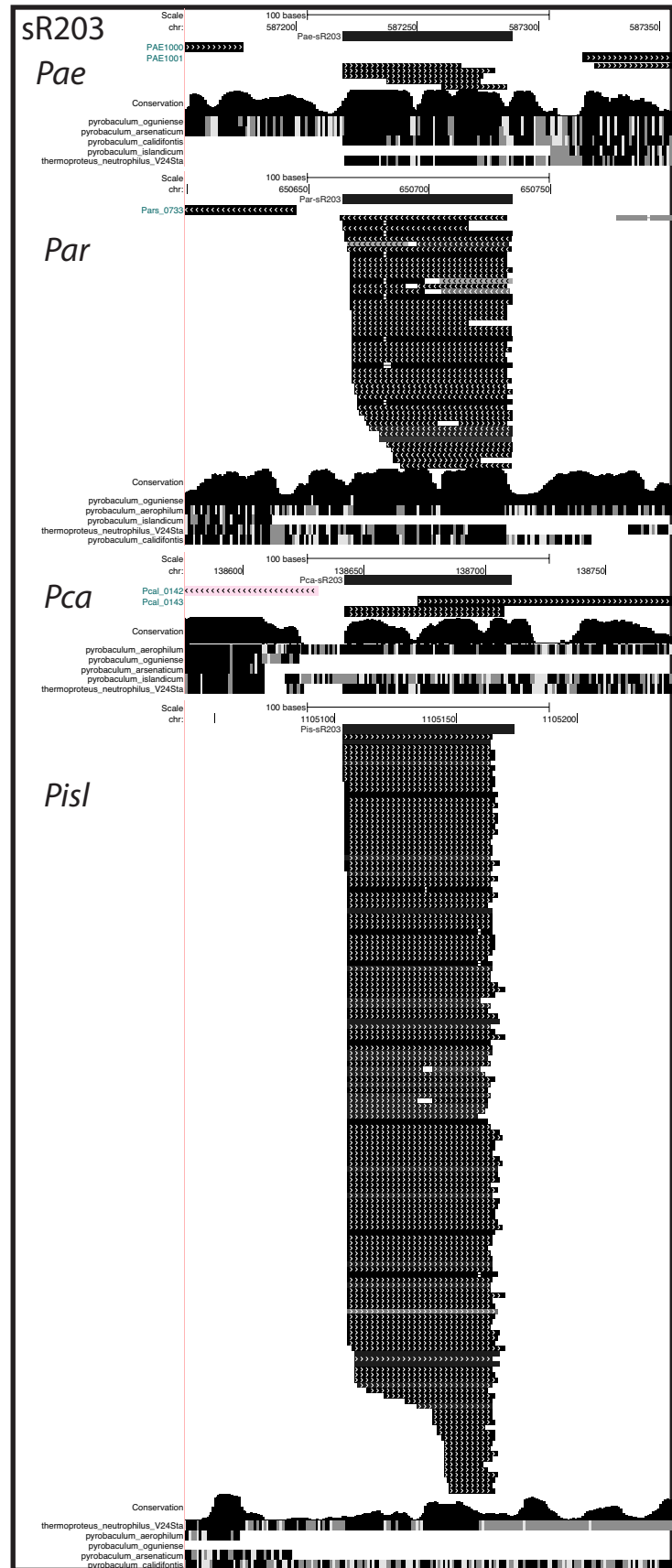
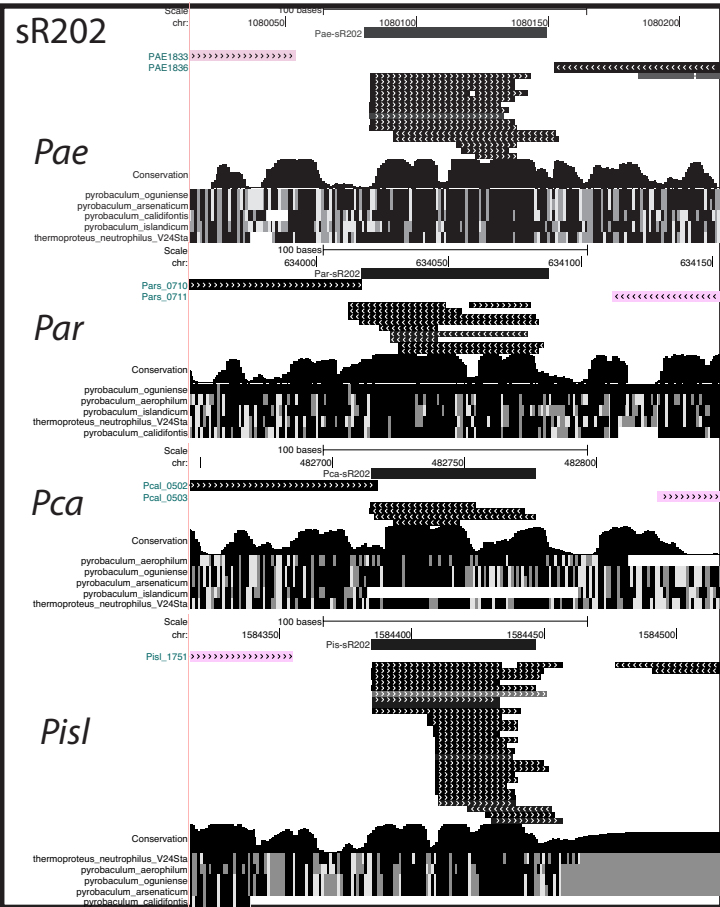


Supplemental Figure 2: Panel A illustrates the alignment of the genomic region in *P. aerophilum* tRNAs upstream of the T-Psi-C motif. The tRNA nucleotides that are complementary to the guide region of sR210 (shown at top) are in red, and anticodons, when present in the alignment, are in purple; the Ψ-Ψ-C (U-Ψ-C) is boxed. Panel B illustrates the sequence logo (weblogo.berkeley.edu) of aligned tRNA nucleotides over the region of guide target interaction.



Supplemental Figure 3: Primer extension Assay in *P. calidifontis*. H/ACA-like sRNA gene products were 5'-end mapped using primer-extension assay in 15% denaturing PAGE. Primers (given below) were annealed to total RNA from *P. calidifontis*, and extended using Superscript III (Invitrogen). Expected lengths of extension products (arrowheads) for sR205, sR206, sR207, sR208 and sR210 are: 51, 51, 57, 57 and 51 nt., respectively. The dominant extension products are consistent with, or shorter (sR207) than expected results derived from sequencing. sR206 (lane 2) extension includes one band above the expected size with associated sequence (gaggTAGA) that may form a four-base 5' guide for 23S-1902 (aacaAUUU), potentially adding a 5' guide region to sR206. However, this unexpected 5' extension is not conserved among the sR206 genes in other *Pyrobaculum* species, so its contribution is unclear given that it appears to co-exist with the shorter form of sR206 with the anticipated 5' boundary. Marker lane (M) - 20/100 ssDNA ladder (Integrated DNA technologies).

Supplemental Figure 4 (following seven pages): RNA sequencing read alignments of H/ACA-like sRNA orthologs for each of the *Pyrobaculum* sRNA species studied, displayed within the UCSC Archaeal Genome Browser (archaea.ucsc.edu). In each genome browser view, horizontal “tracks” display contextual genomic information surrounding sequencing reads (from top to bottom of each panel): genome view scale, chromosome coordinates, predicted gene boundaries of sRNAs (thick black bar with sRNA labels), flanking protein-coding genes (thick bars, color-coded by general functional category; direction of transcription indicated by arrows within bars), sequencing reads (thin black bars; all reads with same start and stop collapsed to single bars), and graph reflecting conservation of multiple sequence alignment among *Pyrobaculum* genomes. Total counts of sequence reads for any locus can be obtained from Supplemental Table 1.



The figure displays four genomic tracks labeled *Pae*, *Par*, *Pca*, and *Pisl*. Each track shows a scale bar at the top indicating a 100 base pair range. Below the scale bar, the gene name and its coordinates are listed. The tracks show conservation scores as black bars and sequence alignments as grey bars. The species compared are *pyrobaculum_oguniense*, *pyrobaculum_arsenicum*, *pyrobaculum_calidifontis*, *pyrobaculum_islandicum*, *thermoproteus_neutrophilus_V24Sta*, and *pyrobaculum_calidifontis*.

- Pae***: Scale chr: 1238250 to 1238350. Gene: *Pae-sR204*. Conservation score: 1238300.
- Par***: Scale chr: 1797450 to 1797550. Gene: *Par-sR204*. Conservation score: 1797500.
- Pca***: Scale chr: 168700 to 168850. Gene: *Pca-sR204*. Conservation score: 168800.
- Pisl***: Scale chr: 1691200 to 1691350. Gene: *Pis-sR204*. Conservation score: 1691300.

SR205

Scale chr: 1001300 100 bases 1001350 1001400 1001450

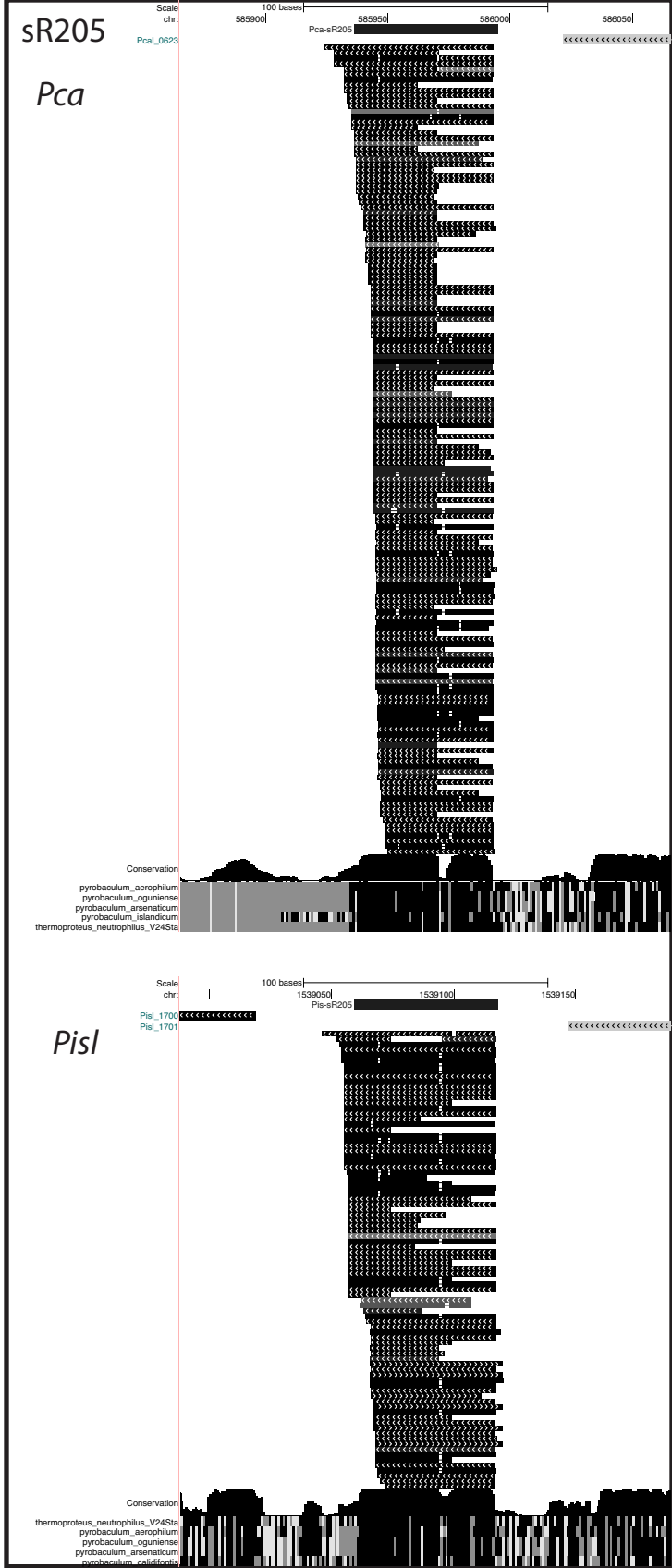
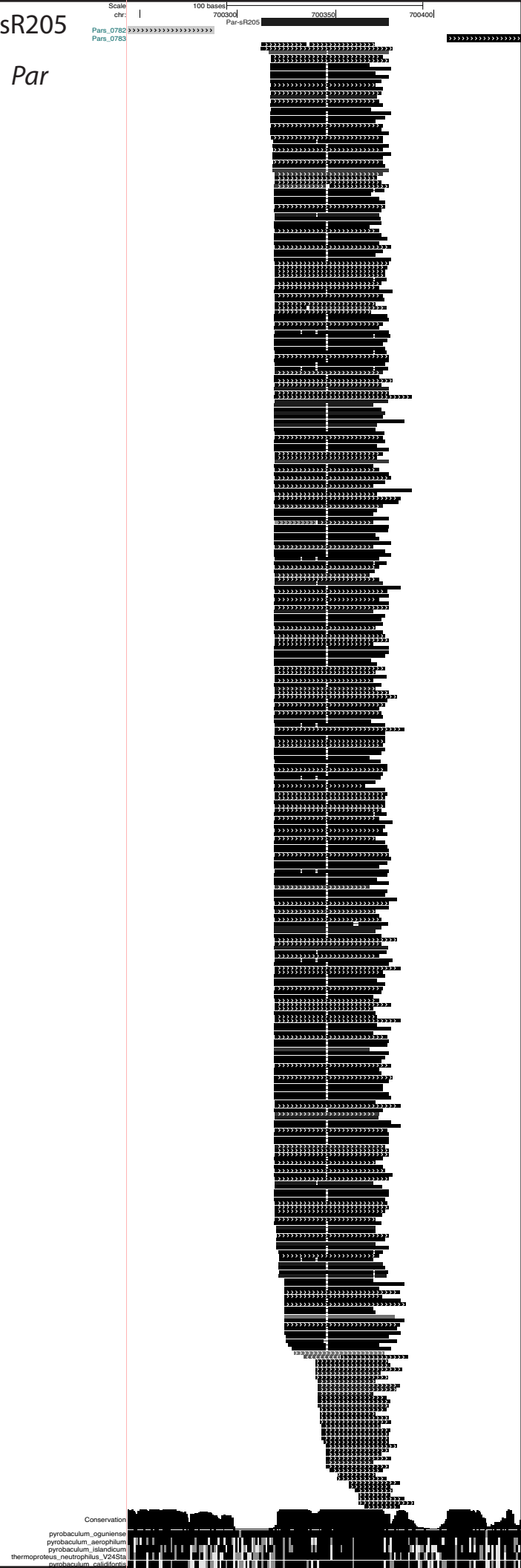
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PAE1715

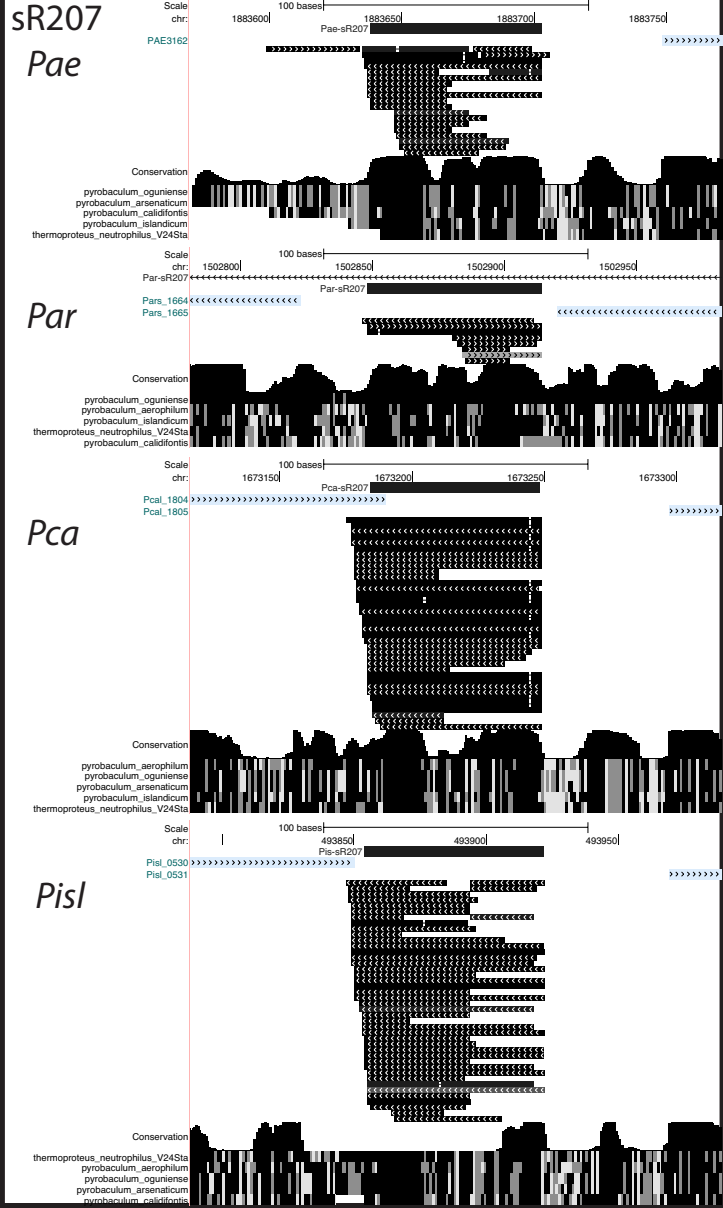
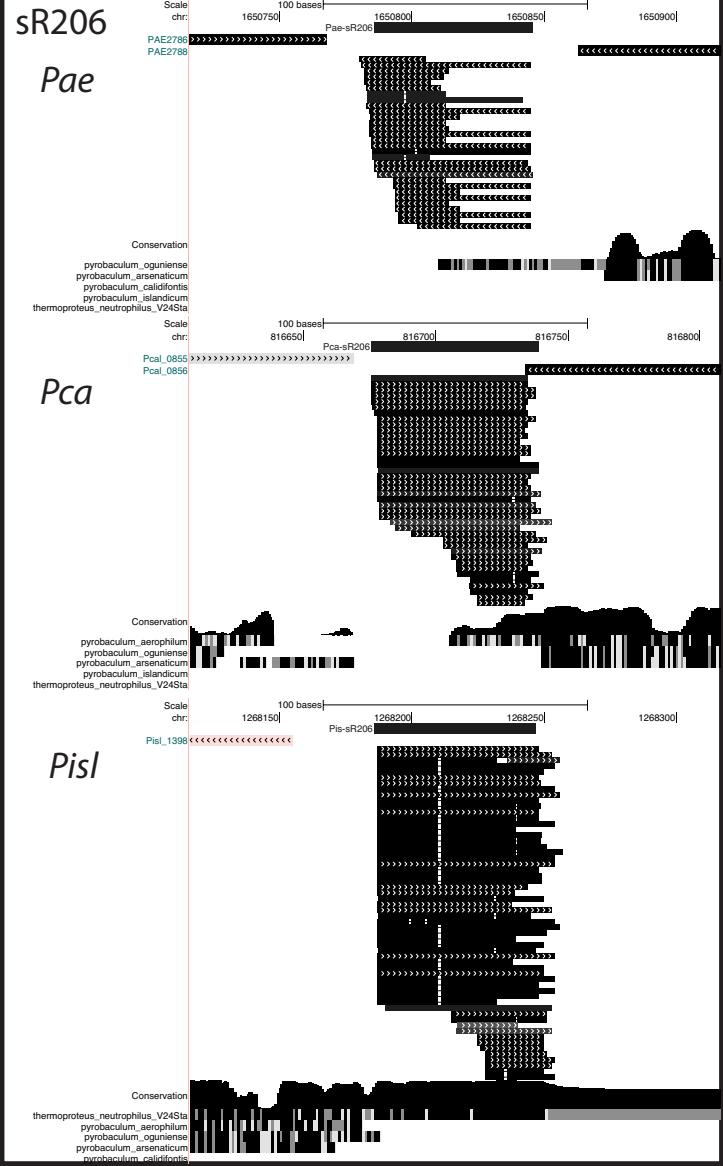
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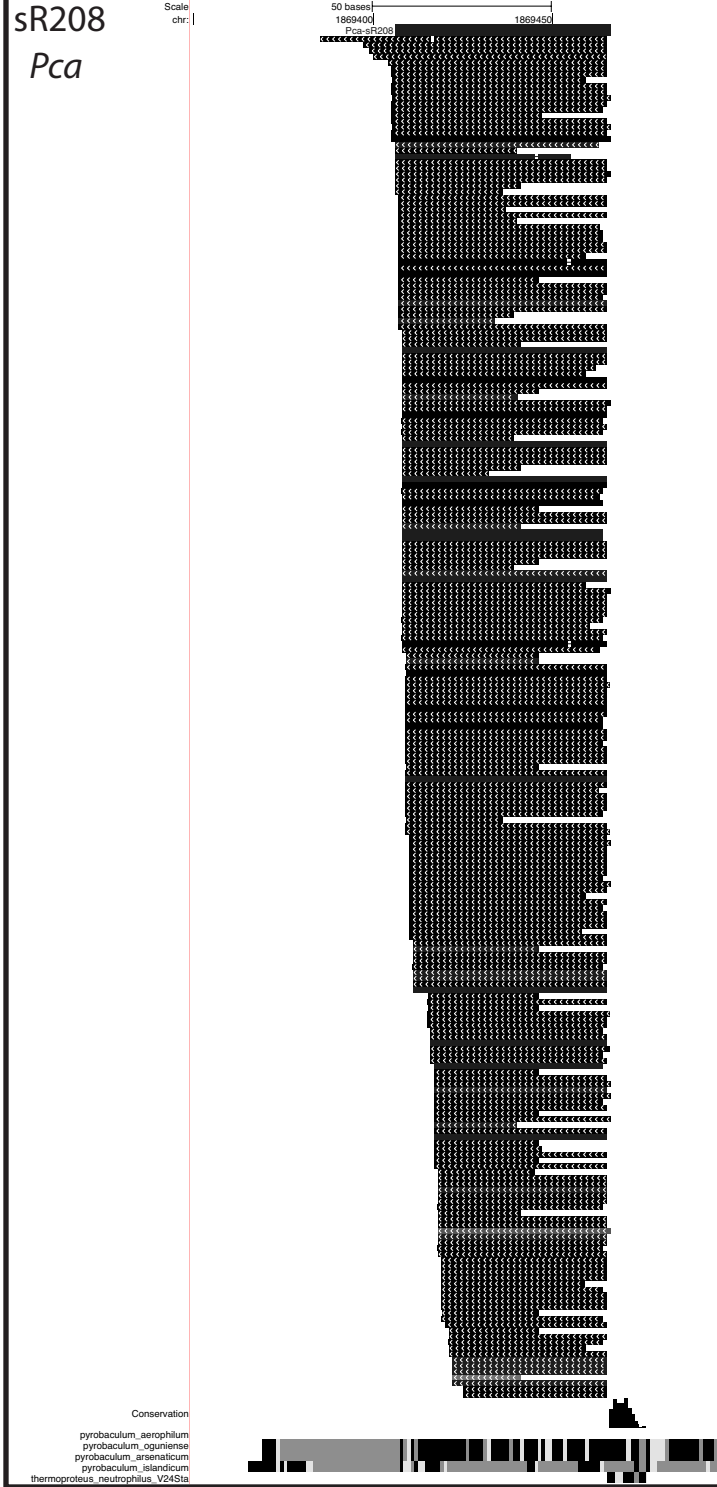
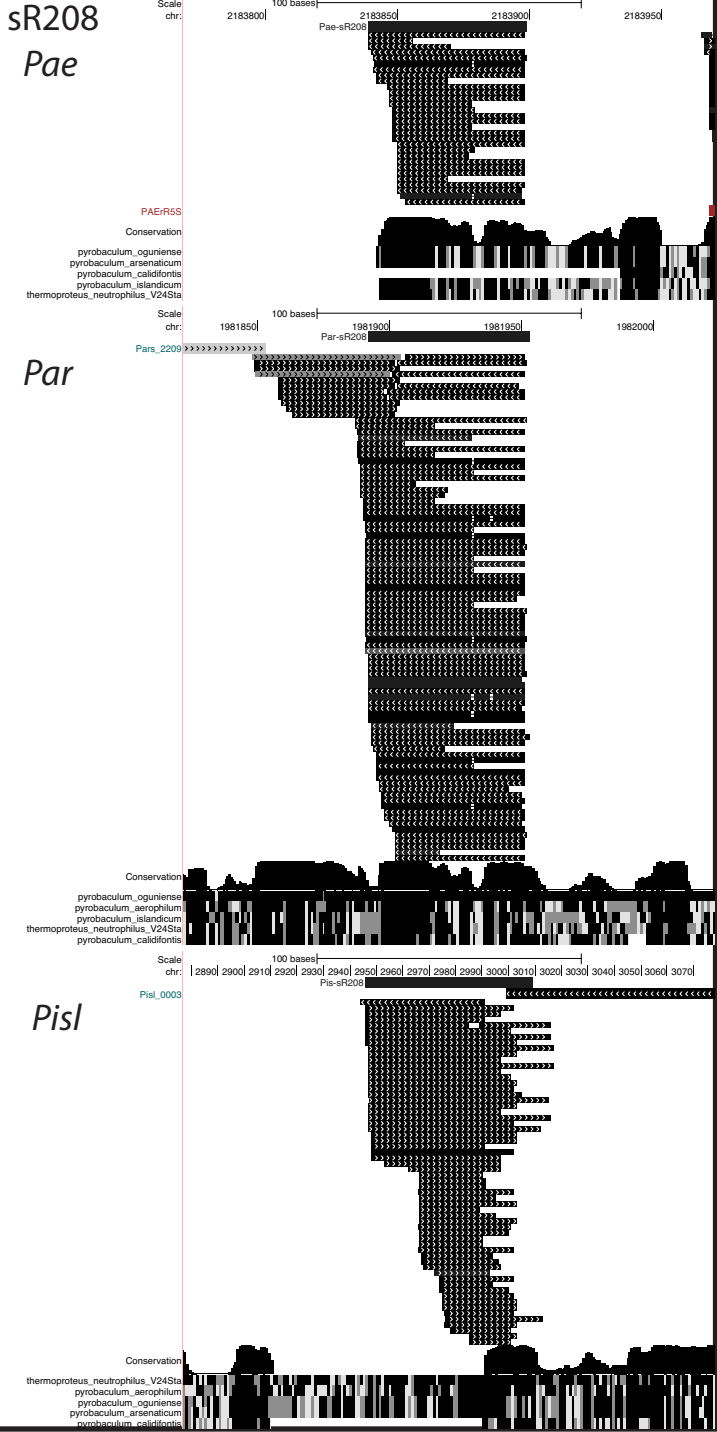
Conservation

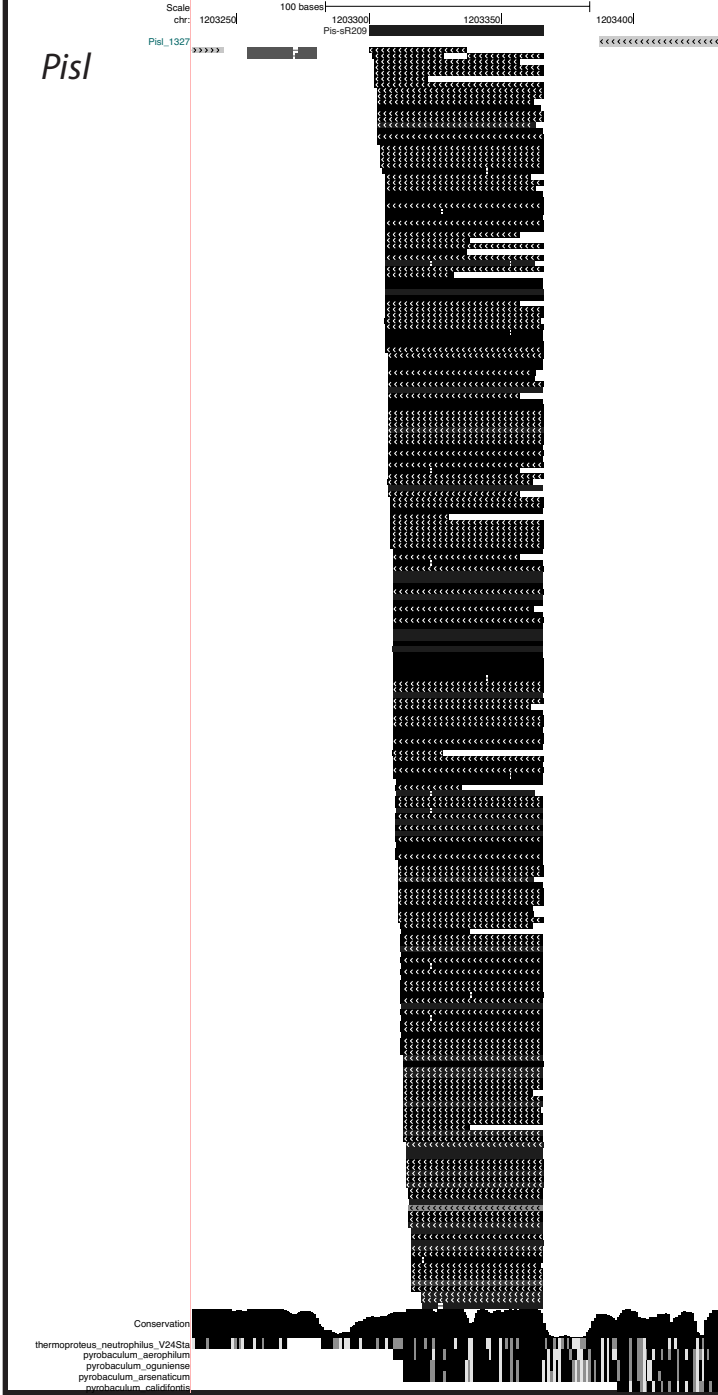
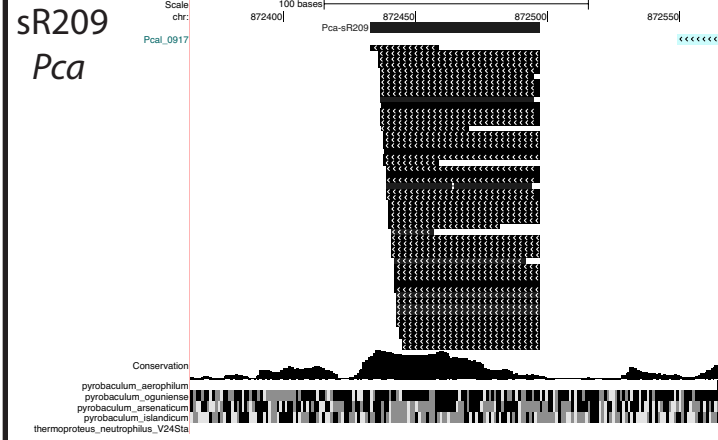
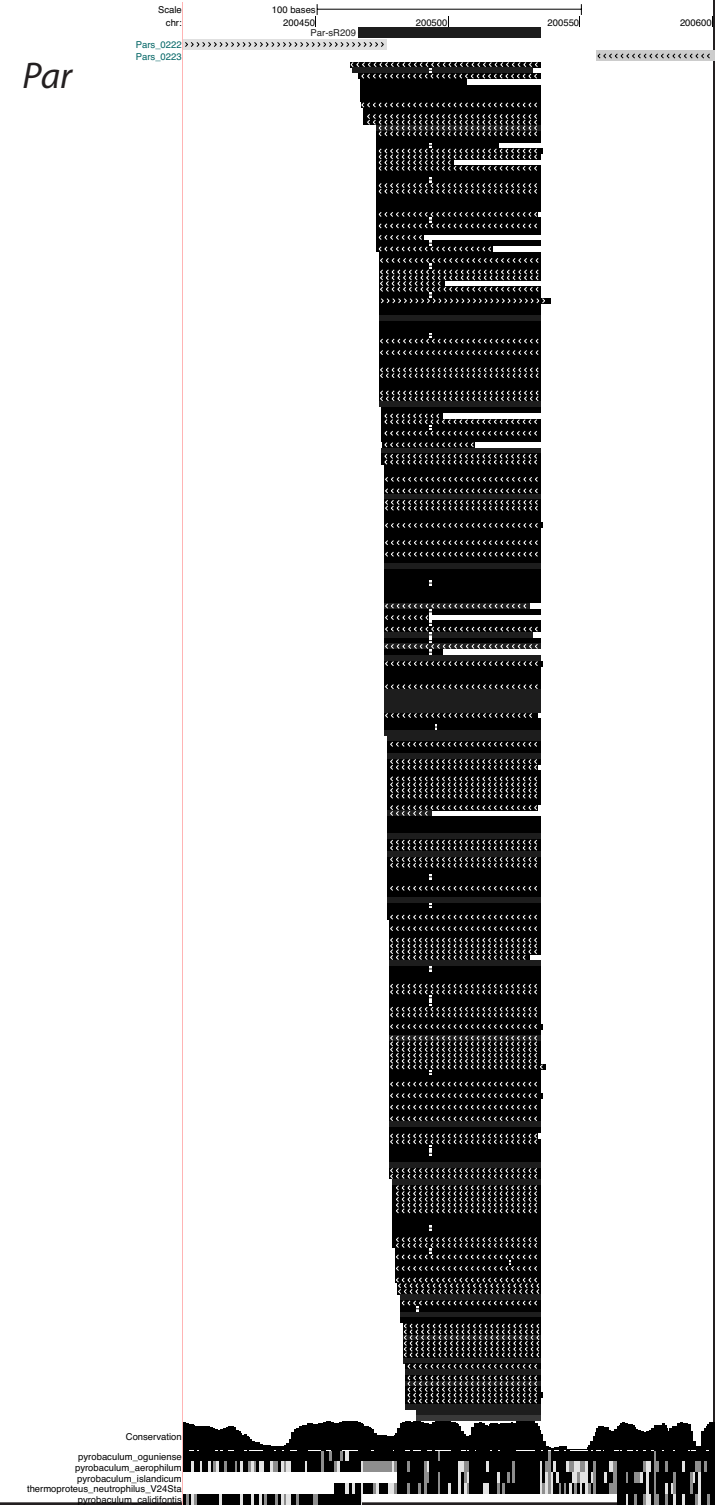
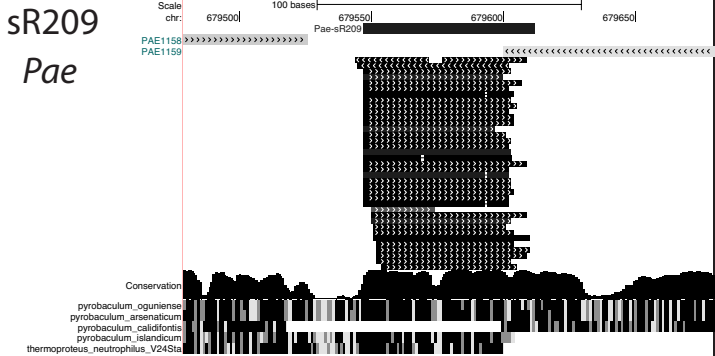
pyrobaculum_oguniense
pyrobaculum_arsenicum
pyrobaculum_caldifortis
pyrobaculum_islandicum
thermoproteus_neutrophilus_V24Sta

1001300 100 bases 1001350 1001400 1001450









Supplemental Table 1: The number of uniquely mapped sequence read for each H/ACA-like gene locus and 5S ribosomal RNA in each species. (n.p) indicates that the orthologous gene locus is not encoded in this species. Number of reads is inclusive of those obtained from both exponential and stationary phase libraries. The numerical results should be interpreted qualitatively (for discovery) since no attempt has been made to normalize across growth conditions or sequencing depth. Read counts for 5S rRNA are included for qualitative comparison.

Gene	<i>P. aerophilum</i>	<i>P. arsenaticum</i>	<i>P. calidifontis</i>	<i>P. islandicum</i>
sR201	3	0	22	11
sR202	29	14	5	34
sR203	7	53	3	447
sR204	11	24	60	59
sR205	458	867	299	113
sR206	35	n.p.	48	71
sR207	24	14	43	63
sR208	36	104	767	99
sR209	141	603	190	810
sR210	354	739	87	66
5S rRNA	709	732	186	40

Primers Used - CMCT assay

Pae-asR201-1091307	CTT CAT CCT GCT GTC GCC
Pae-asR202-1094500	AAA CCG CGG GGT CGA T
Pae-asR203-1091132	CGA CAG CTA GCC CGC AT
Pae-asR205-1093967	AAC TTA CCC GGC AAG GCA T
Pae-asR206-1093841	CCC TTA CGG GCT CGC G
Pae-asR207-1094535	ACC GCC AGG ATG GGA A
Pae-asR208-1093915	TTC AGC GGC GCT TCG
Pae-asR209-1089668	CTT AGC CCT ACC CCG ATA GC

Primers Used - Primer Extension

Pca-asR205	GGC CGG GGT AAC CCG CCA GGC TCA TTC
Pca-asR206	GGG GGA CCC GAC GCG GCT CTT CAC G
Pca-asR207	CCC GCG GCA GGG GGC TCG CTC ATA G
Pca-asR208	GGT GGG CCA CTG GCG TAC GTG GAG TAT TCA
Pca-asR210	CTC GGC CGC GGG CGG GTT TTT CAT

H/ACA-like sRNA Sequences

Note: RNA sequences shown in upper case for sequences recovered in RNA sequencing, lower case is used when sequence was inferred from comparative genomic alignment. Species abbreviations are as follow: *P. aerophilum* (Pae), *P. arsenaticum* (Par), *P. calidifontis* (Pca), *P. islandicum* (Pis), *T. neutrophilus* (Tne), *P. oguniense* (Pog).

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>Pca-sR201
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>Pis-sR201
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>Pae-sR201
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>Par-sR201
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>Tne-sR201

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