Supplementary Figure S1. Relationship of NPKM value of a gene with the TSS score of a promoter upstream of this gene. The log$_{10}$ of both values is plotted. The line gives a linear regression with a regression coefficient of 32%.
Supplementary Figure S2. Schema of the hidden Markov-model for motif discovery. Nodes filled in grey use the same, homogeneous probabilities for the nucleotides, whereas colored nodes correspond to nodes representing motifs with position-dependent probabilities. The offset from the 5’ end of the promoter, the lengths of the motifs $L_1$ and $L_2$, the minimum distance $D$ between the two motifs, and the number of motifs in each component are adjustable by user parameters.
Supplementary Figure S3. Relationship of the TSS and RpoD score. The score of a given TSS was plotted against the score of the RpoD promoter motif upstream of it. The TSS scores with deviations were group into strong (red), medium (orange), weak (yellow) and no RpoD-dependent promoters (blue) and plotted against the RpoD score. Moreover, TSSs with RpoD promoter motifs that were not correctly positioned were also included for upstream sequences containing otherwise strong (purple), medium (light blue) or weak (medium blue) -35 and -10 motifs. The TSS score of three independent biological experiments is shown. The correlation between the values were $R^2 = 0.15$ for the strong (red) RpoD-dependent promoters and $R^2 = -0.035$ for not (blue) RpoD-dependent promoters.
**Supplementary Figure S4.** Relationship of TSS score, RpoD-dependent promoters and expression level of the subsequent genes. For the chromosomal genes in “+” orientation, the NPKM values of the genes in this orientation (black dots) and the TSS score of all TSSs in this orientation (triangles) were plotted against the position. Red triangles indicate strong or medium-strong RpoD-dependent promoters, blue triangles all others.

**Literature cited in the Supplement S1-S4**

1. Große C, Poehlein A, Blank K, Schwarzenberger C, Schleuder G, Herzberg M, Nies DH. 2019. The third pillar of metal homeostasis in *Cupriavidus metallidurans* CH34: Preferences are controlled by extracytoplasmic functions sigma factors. Metallomics 11:291-316.
2. Große C, Grass G, Anton A, Franke S, Navarrete Santos A, Lawley B, Brown NL, Nies DH. 1999. Transcriptional organization of the czc heavy metal homoeostasis determinant from *Alcaligenes eutrophus*. J Bacteriol 181:2385-2393.
3. Große C, Kohl T, Herzberg M, Nies DH. submitted. Loss of mobile genetic elements in *Cupriavidus metallidurans*. 
Supplementary Figure S5. Maps of genetic determinants encoding metal resistances. Maps are shown of determinants in the indicated regions with NPKM values on one DNA strand (red) or the other direction of transcription (blue). Values in orange and light blue are transcripts resulting from multiple homologous DNA regions, which could not be associated to a single locus. Above are the Rmet locus and gene names, the mean NPKM and response values (1). TSSs (flags) are indicated with the corresponding TSS score (not: RpoD score), white for scores < 50 with no promoter consensus motifs indicated, red shades for strong (>1000, red), medium (100-1000, orange) or weak (50-100, light orange) RpoD promoters, blue shades (strong, medium, light) according if not associated to the RpoD model.

Active determinants encoding transenvelope efflux systems for divalent metal cations.

Panel A. The czc determinant on plasmid pMOL30, part 2, the czcP region with Op1817f_1. The czcJ gene and the flgB-czcP operon further downstream from the main czc determinant were again only weakly expressed in TMM-grown cells. The czcJ gene had two weak TSSs 47 and 92 bp upstream, one with a strong RpoD score, the other was no RpoD promoter. The czcP gene for a P$_{ib4}$-type export system had a weak TSS 41 bp upstream not assigned to RpoD. The genes czcP and flgB may be separated by an open reading frame Rmet_6369 on the other DNA strand, which was, however, not expressed.

Panel B. The ancestral czc2 determinant on the chromid; part 1, zntA region. Expression of zntA for the main zinc-exporting P$_{ib2}$-type was expressed with an NPKM value of 48 from a RpoD-dependent medium-strong promoter 12 bp upstream of zntA with sufficient TSS score. The TSS for czcI2C2B2' also started from a RpoD-dependent promoter. This TSS was annotated at position +2 of the czcI2 gene, the second base pair of the AUG start codon. The score for a RpoD promoter responsible for this TSS was only 3.4, however, the search algorithm identified as -35 motif a sequence AATGTTTG at position -39. If instead the
sequence TGTTTG at position -37 was scored, which moved the -35 region also more closely to the ideal TATAAT sequence at position -10, the score increased to 8.40, a strong RpoD score. Consequently, this TSS at +2 of the czcl2 gene started from a RpoD-dependent promoter that was strong enough for the transcript abundance of czcl2. There was no evidence from alignments of czcl genes that czcl2-translation started at a triplet further downstream. Transcription of czcl2 began at a low NPKM value of the 5' end of the gene, increasing to a mean NPKM value of 26, and dropped sharply downstream of czcl2, so that czcC2 and the truncated czcB2' were nearly not expressed in non-amended TMM. Since the clustering window of the nio counts was ±5 bp, the real transcriptional starting point of czcl2 may be close to the +1 position, so that the mRNA may start with the AUG at the 5' end. This would indicate an alternative translation initiation of czcl2 by lmRNA initiation as in case of rpoN. While czcl2 was expressed in TMM-grown cells but not czcC2, both genes were strongly responding to metal stress.

Panel C. The ancestral czc2 determinant on the chromid; part 2, czcR2S2 region. Stars indicate no NPKM value due to multiple homologous regions in the genome. In the other half of czc2, two TSSs mediated a low-level transcription of czcR2S2 with NPKM values of 16 and 8, respectively, which are around the usual cut-off value for NPKM or 10. While the TSS 118 bp upstream of czcR2 was a (w)-sba promoter with weak but not correctly spaced RpoD consensus motifs, the TSS 55 bp upstream of czcR2 displayed a strong RpoD score albeit a low TSS score. Both promoters may be activated under metal stress conditions, for instance by CzcR2. The czcB2''A genes were fused to upstream genes with a strong RpoD-dependent TSS upstream of sulP for a sulfate transporter. The genes were transcribed but should not be translated due to a lacking 5'-end of czcB2''.

Panel D. The cnr determinant on plasmid pMOL28. Transcription of the determinant was initiated from two weak TSSs 966 bp upstream of cnrY and 24 bp upstream of cnrC, and two
medium-strong TSSs 23 bp upstream of cnrY and 763 bp upstream of cnrT, respectively. The medium-strong cnrYp promoter 23 bp upstream of cnrY was a RpoD-dependent promoter with a strong RpoD score while cnrYp 966 bp upstream of cnrY and cnrCp 24 bp upstream of cnrC were no RpoD promoters, which agrees to the fact that cnr expression is under control of the sigma factor CnrH.

Panel E. The zni/zne determinant on the chromid; part 1. The operons zniBA-Rmet_5318 (Op1571r_1), zniC (Op1572f_2), zniS (Op1573r_1) and zniR (Op1574f_1) were expressed in unchallenged C. metallidurans cells and were clearly under control of RpoD-dependent TSSs. The TSS scores were much higher than the NPKM values, indicating the possibility of rapid mRNA degradation. The expression levels in non-challenged cells in combination with the responsiveness to metal stress conditions identified the Zni system as a minor trans-envelope efflux system, which may contribute to metal homeostasis and is under RpoD- and ZniRS control.

Panel F. The zni/zne determinant on the chromid; part 2. The operon zneBAC (Op1577r_1) was also expressed and under RpoD control while only weak, not-RpoD-associated TSSs were upstream of the operons zneRS (Op1576f_1) and zneR2S2 (Op1578f_1). Expression of zneP (Op1576f_1) was very low but the gene was responding to metal stress. It could be transcribed from the RpoD-dependent promoter upstream of zniR and under metal stress also from the weak (white flag in Panel E) but clearly RpoD-dependent promoter within zniR. The weak TSS upstream of zneRS was not RpoD-dependent. Upstream of zneR2S2 were a RpoD and a non-RpoD promoter.
Inactivated determinants

Panel G. The ncc determinant on plasmid pMOL30. No TSS could be identified upstream of this inactivated metal resistance determinant, which agreed to the absence of nccH gene for a ncc-specific sigma factor in C. metallidurans. In contrast to cnr, no RpoD-dependent promoter mediated expression of ncc under non-challenging conditions. A weak TSS 850 bp upstream of nreB could not be associated to RpoD and nreB was not expressed in TMM-grown cell (NPKM = 1) and not responding to metal stress. The determinant was not expressed, contained no TSS and the frame-shift mutation in nccB should result in a premature transcription termination event so that nccA would not be expressed anyhow.

Panel H. The hmv determinant on the chromid. Operon Op1066f_2 on the chromid with hmvCBA’/A'' has the hmvA gene interrupted by a frame-shift mutation. It started from a strong TSS 181 bp upstream and a medium TSS 71 bp upstream of hmvC and both TSSs could be associated to RpoD. Two flanking operons on the same DNA strand started from TSSs, which also could be assigned to the RpoD model.
Panel I. The hmy determinant on the chromid. The hmy determinant on the chromid was split into two halves by a transposon insertion between hmyB and hmyA, which inactivates the genes. The first half is Op1160f_1 with hmyFCB and a strong expression of hmyF from a strong and a medium strong TSS, which could both not be associated to RpoD. The hmyA gene showed some expression (NPKM = 19) but no TSS. Expression could be the result of a read-through from hmyB.

Panel J. The nim determinant on the chromid. The nim determinant on the chromid is adjacent to a copper resistance cluster and also interrupted by a transposon insertion into the nimA gene. The nim genes were expressed, TSS were identified but could not be associated to RpoD. A strong RpoD-associated TSS started transcription of a pilus synthesis operon on the other DNA strand.
Panel K. The *hmz* determinant on the chromosomal island CMGI-4. A last determinant was *hmz* and located on the chromosome, again with a transposon adjacent. While *hmzBA* were nearly not expressed and no TSS could be identified, the regulatory genes *hmzRS* was expressed from a RpoD-dependent TSS.

Transenvelope efflux systems involved in copper resistance.

Panel L. The *cus* determinant on the chromid. The *cusDCBA* determinant operon Op1480f.2 on the chromid was not expressed in non-challenged cells with the exception of the *cusD* gene at the 5' end, which encodes an uncharacterized protein. This operon was under control of a strong TSS upstream of *cusD* and this TSS could be clearly associated to the RpoD model. The expression level of *cusCBAF* was very low with NPKM values of 3, 1, 1, 5 respectively, although the TSS representing the *cusDp* promoter had a strong TSS score of 2235 and yielded a *cusD* transcript with NPKM = 42. A terminator may be located downstream of *cusD* but this would not explain the small NPKM value of *cusD* with respect to the strong TSS score of its promoter. Alternatively, transcription from two promoters downstream of the *cus* operon on the other DNA strand may yield an antisense transcript that de-stabilizes the *cusDCBAF* transcript. This would be the first example of an antisense regulation in *C. metallidurans*. The scores of the TSSs in the antisense direction would be strong enough to explain such an event. Both were non-RpoD-promoters, so that another sigma factor should be involved here.
Panel M. The sil determinant on plasmid pMOL30. The second determinant for a copper-exporting transenvelope system is silDCBA in operon Op1874r_1 on plasmid pMOL30, which was also weakly expressed in unchallenged cells, again with the exception of the cusD paralog silD. The sil genes were not responding to metal stress but silver was not part of the challenging metals. Two TSSs were upstream of silD and strong enough for sil expression, one of these could be associated to the RpoD model.

Other transition metal resistance determinants.

Panel N. The cadA determinant on the chromosomal island CMG-1. The chromosomal cad determinant, composed of the cadR gene for the MerR-type regulator (2) in one direction of transcription and cadAC for the CadA PIB2-type ATPase and protein CadC, started from two RpoD-dependent TSSs in a common promoter region, a strong TSS for cadR and a weak TSS for cadA, which was interestingly 9 base pairs downstream of the annotated cadA ATG. This indicated a later translation initiation start site, e.g. at the ATG 14 triplets further downstream than the annotated ATG, although a respectively truncated CadA protein (CadA3) mediated no cadmium resistance in *Escherichia coli* compared to CadA2 as annotated (3). An explanation could be that cadA3 expression in *C. metallidurans* occurs as lTRNA initiation but *E. coli* was not able to translate such a message. The cad determinant was strongly responding to metal stress and under control of RpoD and CadR.
Panel O. The *pbr* determinant on plasmid pMOL30. The *pbr* lead resistance region on pMOL30 is organized as divergonic operons *pbrAB/CD* (Op1808r_1) and *pbrRTU* (Op1807f_1) with a transposon insertion interrupting *pbrU*. The *pbrAB/CD* genes were expressed in TMM-grown cells and responding to metal stress, *pbrRT* expressed but not responding. Only weak TSSs could be associated to *pbrAB/CD* for the P\(_{IB2}\)-type ATPase PbrA, a putative periplasmic protein PbrC/D, which is organized as two polypeptides in other bacteria, and a lead-binding protein PbrD (4), indicating a repressed operon in the non-challenged cells. The *pbrAp, pbrRp, pbrTp* and *pbrB/Cp* promoters displayed a strong RpoD consensus score. While transcriptional initiation activity from *pbrAp* and *pbrB/Cp* was below a TSS score of 50, activity of the TSSs 21 bp upstream of the gene for the MerR-type regulator *pbrR* and 39 bp upstream of *pbrT*, annotated as lead uptake protein, respectively, were above a TSS score of 200 (4-6). Lead resistance was under RpoD control, PbrR and PbrT may be present in the cells to watch out for lead ions so that the *pbr* determinant can be activated when needed.

Panel P. The *cop* determinant on plasmid pMOL30, part 1. The huge multi-gene copper resistance region *cop* on plasmid pMOL30 is organized in nine operons (also Suppl. Fig. 6Q): (i) Op1871r_1 with *copW* but no TSS; (ii) Op1870r_1 with *copE* and also no TSS found; (iii) Op1869f_1 with *copHQ* without TSS; (iv) Op1868r_1 with *copL* and a weak TSS upstream; (v) Op1867f_1 with *copOFGJ* with weak TSSs 661 bp upstream of *copO* and 101 bp upstream of *copF*; (vi) Op1866r_1 with *copA1B1C1D1J* in a clear pentacistronic operon with three weak TSSs upstream of *copA1*; (vii) Op1865f_1 with *copR1S1N* tricistronic with several weak TSS far upstream of this region; (viii) Op1864r_1 with *copMK* with a strong RpoD-promoter 2.3 kb upstream of *copM*; and (ix) Op1863r_1 with *copTV* and a RpoD-dependent promoter 22 bp upstream of the *copT* gene. Main products of this large region at the copper-exporting P\(_{IB1}\)-type ATPase CopF and the periplasmic Cu(I)-oxidase CopA1 (7). One of the three TSSs upstream of *copA1* displayed a strong RpoD motif but low TSS score, indicating a repressed state. The other two TSSs were none RpoD-dependent and scoring as “(m)-sba”, indicating a
possible influence of other sigma factors on copA1 expression. The copFp promoter was clearly RpoD-dependent but on a low level of activity. Expression level of all these genes was very low in unchallenged cells, between NPKM 3 for copH to 65 for copG, corresponding to the fact that this region mediates a high level of copper resistance but only in copper-induced C. metallidurans cells (8), as was also demonstrated by the response values of the cop genes. Reminiscent to cus, two strong RpoD-associated TSSs started transcription in an anti-sense direction of copOFGJ, which may help to repress the determinant when not needed. Otherwise, the two-component regulatory system CopR1S1 may be responsible for cop activation.

Panel Q. The cop determinant on plasmid pMOL30, part 2. The copR1p upstream promoter with a TSS score of 28 is clearly a non-RpoD-promoter so that another sigma factor may be responsible for expression of the cop activator. This cop determinant on plasmid pMOL30 was under control of RpoD, at least one other sigma factor, probably of CopR1S1, control of copR1S1 by a non-RpoD sigma factor, and a possible antisense action on copF expression under RpoD control.

Panel R. The cup determinant on the chromosome. Two other determinants involved in copper resistance in C. metallidurans are the cup determinant for the P_{IB1}-type ATPase CupA on the chromosome outside of any chromosomal island and the cop2 determinant around the CopA2 periplasmic Cu(I) oxidase on the chromid shown in Panel S. Operon Op0974-1 with cupAR was transcribed from a non-RpoD-associated, sufficiently strong TSS 38 bp upstream of cupA, while no TSS was found upstream of Op0975f_1 cupC, despite an expression level of NPKM = 76. Expression of cupAR is probably regulated by CupR (9) but again, a non-RpoD sigma factor seems to be required for cup expression.
Panel S. The cop2 determinant on the chromid. The cop2 determinant on the chromid is organized in the two divergently oriented operons. Op1695r_1/2 with copA2B2C2D2 was nearly not expressed and no TSS was found. The operon is clearly responding to metal stress so that the lack of an identified TSS was probably due to a lack of transcription. Op1696f_1 with copR2S2 for a two-component regulatory system started from three weakly scoring TSSs. A promoter 147 bp upstream of copR2 possessed a strong RpoD consensus, the promoters 75 bp and 46 bp upstream were non-RpoD promoters. The genes copR2S2 may be expressed by RpoD and at least one other sigma factor.

Panel T. The chr determinant on plasmid pMOL28. The chr chromate resistance determinant on plasmid pMOL28 was organized as deca-cistronic operon around the chrA1 gene for a chromate efflux pump (10-12) and clearly under RpoD control. Since chromate was not part of the metal cocktail challenging C. metallidurans strains (1), chr was not responding and served as negative control here. No TSSs for the divergently transcribed chrI gene for a possible regulator was in the direct vicinity of chrI, the closest TSS was 1553 bp upstream within chrA1, not associated to RpoD and had a TSS score of 61±16 (Suppl. Data Set 1; TSS not shown in the figure above). A possible CnrH-dependent promoter for the adjacent cnrYXH genes was within the chrI genes and 43 bp downstream of the chrI start codon. Should chrI start later than predicted, the gene may be expressed together with cnr under CnrH and nickel control. The chr genes were nearly not expressed in non-challenged cells with NPKM values between 3 and 12.
Panel U. The chr2 determinant on the chromid. Expression of a second chr2 resistance determinant Op1075r_2 on the chromid was even lower than that of chr, between NPKM 1 and 2 but also from an TSS with a RpoD motif upstream. In case of chrB1 on pMOL28 (Panel T) and the open reading frame Rmet_3867 upstream of chrB2 on the chromid, the distance between the TSS and the start codon was 0, again indicating alternative translation initiation by lmrna initiation (13). Chromate resistance was under RpoD control with a remote possibility of a contribution of CnrH via ChrI.

Panel V. The ars determinant on the chromosomal island CMGI-7. The arsRIC2BC1HP determinant Op0094r_2/3 on the chromosomal island CMGI-7 was on an expression level between NPKM 5 and 25, also from a TSS immediately upstream of arsR, which was RpoD-dependent. The respective transcript should start with the start codon of arsR, indicating again lmrna-specific translation initiation (13). The arsM gene for an arsenate methyl-transferase upstream of arsR was the last gene of the tetracistronic operon Op0094r_1 upstream of the arsRIC2BC1HP operon. Transcription started from a sufficiently strong but not RpoD-associated TSS 20 bp upstream of Rmet_0337 but only this gene and Rmet_0336 downstream exhibited some level of expression. Again, while most genes were under RpoD control, genes that may enlarge the capability of arsenate resistance in C. metallidurans, for instance by allowing arsenate methylation, were under non-RpoD control.
Panel W. The *mer* determinant on the chromosome. Expression of the *mer* determinant on the chromosome was so low that no open reading frames could be assigned to the RNASeq sequences, and only one weak TSS was found upstream of the *merR* gene.

Panel X. The *mer* determinant on plasmid pMOL28. Transcripts of the *mer* determinant on plasmid pMOL28 could not be assigned to this region due to the high degree of homology between this and the *mer* determinant on pMOL30.

Panel Y. The *mer* determinant on plasmid pMOL30, part 1. A *merP* gene apart from the *merTPADE* region on plasmid pMOL30 (but nevertheless on plasmid pMOL30) was under RpoD control.
Panel Z. The *mer* determinant on plasmid pMOL30, part 2. Transcripts of this part of the *mer* determinant on plasmid pMOL30 could not be assigned to this region due to the high degree of homology between this and the *mer* determinant on pMOL28.

**Literature for this figure.**

1. Große C, Kohl T, Herzberg M, Nies DH. 2022. Loss of mobile genomic islands in metal resistant, hydrogen-oxidizing *Cupriavidus metallidurans*. Appl Environ Microbiol 88:e02048-21.
2. Schulz V, Schmidt-Vogler C, Strohmeyer P, Weber S, Kleemann D, Nies DH, Herzberg M. 2021. Behind the shield of Czc: ZntR controls expression of the gene for the zinc-exporting P-type ATPase ZntA in *Cupriavidus metallidurans*. J Bacteriol 203:e00052-21.
3. Scherer J, Nies DH. 2009. CzcP is a novel efflux system contributing to transition metal resistance in *Cupriavidus metallidurans* CH34. Mol Microbiol 73:601-621.
4. Borremans B, Hobman JL, Provoost A, Brown NL, Van der Lelie D. 2001. Cloning and functional analysis of the *pbr* lead resistance determinant of *Ralstonia metallidurans* CH34. J Bacteriol 183:5651-5658.
5. Taghavi S, Lesaulnier C, Monchy S, Wattiez R, Mergeay M, van der Lelie D. 2009. Lead(II) resistance in *Cupriavidus metallidurans* CH34: interplay between plasmid and chromosomally-located functions. Antonie Van Leeuwenhoek 96:171-182.
6. Debut AJ, Dumay QC, Barabote RD, Saier MH. 2006. The iron/lead transporter superfamilly of Fe³⁺/Pb²⁺ uptake systems. J Mol Microbiol Biotechnol 11:1-9.
7. Monchy S, Benotmane MA, Wattiez R, van Aelst S, Auquier V, Borremans B, Mergeay M, Taghavi S, van der Lelie D, Vallaeyes T. 2006. Transcriptomics and proteomic analysis of the pMOL30-encoded copper resistance in *Cupriavidus metallidurans* strain CH34. Microbiology 152:1765-1776.
8. Wiesemann N, Bütof L, Herzberg M, Hause G, Berthold L, Etschmann B, Brugger J, Martinéz-Criado G, Dobritzsch D, Baginski S, Reith F, Nies DH. 2017. Synergistic toxicity of copper and gold compounds in *Cupriavidus metallidurans* Appl Environ Microbiol 83:e01679-17.
9. Jian X, Wasinger EC, Lockard JV, Chen LX, He C. 2009. Highly sensitive and selective gold(I) recognition by a metalloregulator in *Ralstonia metallidurans*. J Am Chem Soc 131:10869-10871.
10. Juhnke S, Peitzsch N, Hübener N, Große C, Nies DH. 2002. New genes involved in chromate resistance in *Ralstonia metallidurans* strain CH34. Arch Microbiol 179:15-25.
11. Nies DH, Koch S, Wachi S, Peitzsch N, Saier MHJ. 1998. CHR, a novel family of prokaryotic proton motive force-driven transporters probably containing chromate/sulfate transporters. J Bacteriol 180:5799-5802.
12. Nies A, Nies DH, Silver S. 1990. Nucleotide sequence and expression of a plasmid-encoded chromate resistance determinant from *Alcaligenes eutrophus*. J Biol Chem 265:5648-5653.
13. Yamamoto H, Wittek D, Gupta R, Qin B, Ueda T, Krause R, Yamamoto K, Albrecht R, Pech M, Niehaus KH. 2016. 70S-scanning initiation is a novel and frequent initiation mode of ribosomal translation in bacteria. Proc Natl Acad Sci U S A 113:E1180-E1189.
Supplementary Figure S6. Maps of chromosomal genetic determinants encoding genes required for autotrophic growth. Maps are shown of determinants in the indicated regions with NPKM values on one DNA strand (red) or the other direction of transcription (blue). Values in orange and light blue are transcripts resulting from multiple homologous DNA regions, which could not be associated to a single locus. Above are the Rmet locus and gene names, the mean NPKM and response values (3). TSSs (flags) are indicated with the corresponding TSS score, white for scores < 50 that were not assigned to promoter consensus motifs, red shades for strong (>1000, red), medium (100-1000, orange) or weak (50-100, light orange) RpoD promoters, blue shades (strong, medium, light) according if not associated to the RpoD model. The region for the soluble hydrogenase was annotated by TraV-Mac as integrative element as one long red bar, so that the positions of the open reading frames were added manually for one direction of transcription (green arrows).

Panels A (top) and B (bottom). The predicted operon Op0370r_1 on chromosomal island CMGI-3 encoding the membrane-bound hydrogenase. The genes for synthesis of a membrane-bound hydrogenase are all in one large multicistronic operon Op0370r_1 and transcribed from one sufficiently strong TSS that could not be associated to RpoD (Panel B). After 28 genes were transcribed in this operon, transcription may even continue across an inserted transposon (Panel A), indicating the ability of the C. metallidurans RNA polymerase to transcribe more than 18.5 kb of DNA. The responsible TSS was 56 bp upstream of hoxK, the first gene of this large operon. The pattern recognition software identified a 4,1 model upstream of this TSS with a GCCCGAGGC at position -41 and a CGTACATTGT at -13 with the highly conserved second “A” and last “T” of the typical TATAAT -35 site conserved (Suppl. Table S1). The RpoD score was -4.6 so that a sigma factor different from RpoD, which uses nevertheless a -10 site similar to that of RpoD, may be responsible for expression of this large operon. A non-RpoD-dependent promoter may initiate antisense-transcription of the operon.
Panel C. The region on chromosomal island CMGI-2 encoding the soluble hydrogenase, part 1. Arrangement of the genes for the Calvin cycle enzymes and a soluble, NADH-reducing hydrogenase was far more complicated than that of the genes for the membrane-bound hydrogenase. These genes were arranged in six operon regions Op0422r to Op0427f (Panels C to H). Transposon insertions flank this gene cluster, which seem to have been integrated into a ccbA gene for a fructose-1,6-bisphosphate aldolase, with the cbbA1 and a cbbA∆ gene fragment still located at the flanks of the cluster. Additional transposon insertions re-arranged Op0423f and Op0424r, and interrupted the pntAA gene to separate the regions Op0425f and Op0427f from each other. The operon Op0422r_1 cbbLSQOY for the ribulose-bis-phosphate carboxylase and other enzymes was expressed and the weakly scoring TSSs (26±7) was under control of a medium-strong RpoD promoter, which was not strong enough to explain the NPKM value of the first gene, cbbL. A RpoD-associated TSS initiated transcription of cbbR1 for a regulatory protein in operon Op0423f_1, 1 bp downstream of the start codon of this gene, indicating again mRNA-type translation initiation (1).

Panel D. The region encoding the soluble hydrogenase, part 2. A strong, RpoD-dependent promoters upstream of cbbF1 may be responsible for transcription events than continue into the cbbLSQOY region (Panel C) and may have been followed by differential mRNA stability concerning the middle part of the transcript (2). A weakly scoring TSS associated to RpoD initiated transcription of Op0424r_1 with cbbR2 for a regulatory protein and Op0424r_2/3 with cbbF1 and the transposase. The gene expression levels downstream of the strong RpoD-dependent promoter did not match the TSS score of this promoter. This indicated that these promoters may not be responsible for expression of cbbLS (Panel C), in combination with differential mRNA stability. A TSS associated with RpoD was responsible for transcription initiation of Op0425f_1 cbbSAE1F2PT1Z1G1-pgk1-cbbYA2-pykA2JiI1 containing genes for additional Calvin cycle enzymes (continues in Panel E).
Panel E. The region encoding the soluble hydrogenase, part 3. A TSS associated with RpoD (Panel C) was responsible for transcription initiation of Op0425f_1 \( cbbS\Delta E1F2PT1Z1G1-pgk1-cbbYA2-pykA2JiI1 \) containing genes for additional Calvin cycle enzymes and Op0425f_2 containing \( hoxFUYHWI \) genes for hydrogenase maturation and synthesis (continues in Panel F).

Panel F. The region encoding the soluble hydrogenase, part 4. A TSS associated with RpoD (Panel C) was responsible for transcription initiation of Op0425f_1 \( cbbS\Delta E1F2PT1Z1G1-pgk1-cbbYA2-pykA2JiI1 \) containing genes for additional Calvin cycle enzymes and Op0425f_2 containing \( hoxFUYHWI \) genes for hydrogenase maturation and synthesis.

Panel G. The region encoding the soluble hydrogenase, part 5. A putative ancestral operon Op0425f-Op0427f seems to have been interrupted by transposon insertion into a \( pntAA \) gene.
for an alanine dehydrogenase. The transposase gene is now region Op0426r. The pntAA''/AB/B genes for the remaining components of the dehydrogenase were on a low expression level in operon Op0427f_1 (Panel F). One TSS associated to RpoD initiated transcription of hoxN for a nickel importer in Op0427f_2 on the same DNA strand.

Panel H. The region encoding the soluble hydrogenase, part 6. A very strong and RpoD-associated TSS initiated transcription of the main structural genes hypA2B2F2C2D2E2XA' for the soluble hydrogenase on Op0427f_4.

**Literature for this figure.**

1. Yamamoto H, Wittek D, Gupta R, Qin B, Ueda T, Krause R, Yamamoto K, Albrecht R, Pech M, Nierhaus KH. 2016. 70S-scanning initiation is a novel and frequent initiation mode of ribosomal translation in bacteria. Proc Natl Acad Sci U S A 113:E1180-E1189.
2. Owolabi JB, Rosen BP. 1990. Differential mRNA stability controls relative gene expression within the plasmid-encoded arsenical resistance operon. J Bacteriol 172:2367 2371.
Supplementary Table S1. Transcriptional start points in the transcriptome of TMM-grown Cupriavidus metallidurans CH34 cells. The TSS were indexed according to the mean value of the TSS determination score, which is indicated (Mean) with the position, sequence, score and LRR. All values are shown as the mean of biological replicates and within the standard deviation (SDEV). Significant P-values (FDR < 0.05) are indicated with an asterisk (*). The TSS were indexed according to the mean value of the TSS determination score, which is indicated (Mean) with the position, sequence, score and LRR. All values are shown as the mean of biological replicates and within the standard deviation (SDEV). Significant P-values (FDR < 0.05) are indicated with an asterisk (*).
| Gene | Start | Length | Expression | Function |
|------|-------|--------|------------|----------|
| TSS_298217+2 | 612 | 133 | 0.1 | -16 |
| TSS_panel_1001468+1 | 610 | 25 | 0.1 | -14 |
| TSS_116804+3 | 609 | 56 | 0.1 | -37 |
| TSS_312650+2 | 606 | 61 | 0.1 | -13 |
| TSS_201719+5 | 606 | 78 | 0.1 | -13 |
| TSS_405397+3 | 607 | 118 | 0.1 | -15 |
| TSS_877359+3 | 605 | 75 | 0.1 | -15 |
| TSS_230979+4 | 605 | 19 | 0.1 | -15 |
| TSS_44444+2 | 604 | 77 | 0.1 | -17 |
| TSS_503397+3 | 602 | 114 | 0.1 | -16 |
| TSS_249557+2 | 601 | 76 | 0.1 | -12 |
| TSS_2631643+2 | 599 | 157 | 0.1 | -15 |
| TSS_7685150+2 | 598 | 61 | 0.1 | -19 |
| TSS_3150024+2 | 598 | 94 | 0.1 | -18 |
| TSS_2064999+3 | 597 | 51 | 0.1 | -17 |
| TSS_770363+2 | 597 | 55 | 0.1 | -14 |
| TSS_171427+2 | 594 | 103 | 0.1 | -15 |
| TSS_2357590+2 | 594 | 116 | 0.1 | -11 |
| TSS_43132+2 | 592 | 95 | 0.1 | -16 |
| TSS_563649+3 | 588 | 143 | 0.1 | -17 |
| TSS_118297+2 | 586 | 64 | 0.1 | -16 |
| TSS_759811+3 | 585 | 49 | 0.1 | -15 |
| TSS_487426+2 | 583 | 576 | 0.1 | -15 |
| TSS_341657+5 | 581 | 41 | 0.2 | -16 |
| TSS_189034+2 | 579 | 70 | 0.1 | -16 |
| TSS_1272795+3 | 578 | 53 | 0.1 | -25 |
| TSS_238310+3 | 577 | 85 | 0.1 | -16 |
| TSS_355652+2 | 577 | 58 | 0.1 | -25 |
| TSS_607335+2 | 574 | 87 | 0.1 | -13 |
| TSS_392078+2 | 573 | 47 | 0.1 | -27 |
| TSS_2796668+2 | 572 | 70 | 0.1 | -17 |
| TSS_189467+2 | 571 | 53 | 0.1 | -16 |
| TSS_129768+3 | 570 | 95 | 0.1 | -15 |
| TSS_241163+2 | 569 | 119 | 0.1 | -16 |
| TSS_459260+3 | 569 | 136 | 0.1 | -16 |
| TSS_2063461+3 | 568 | 39 | 0.1 | -16 |
| TSS_238017+2 | 568 | 133 | 0.1 | -16 |
| TSS_3233485+2 | 568 | 173 | 0.1 | -13 |
| TSS_3710506+2 | 568 | 15 | 0.1 | -15 |
| TSS_788219+2 | 567 | 87 | 0.1 | -15 |
| TSS_2770540+2 | 566 | 416 | 0.1 | -42 |
| TSS_447500+2 | 565 | 49 | 0.1 | -16 |
| TSS_527162+2 | 564 | 153 | 0.1 | -13 |
| TSS_panel_4852964+1 | 564 | 94 | 0.1 | -16 |
| TSS_1715166+2 | 564 | 107 | 0.1 | -17 |
| TSS_3412562+6 | 564 | 132 | 0.1 | -27 |
| TSS_panel_4855665+1 | 562 | 74 | 0.1 | -5 |
| TSS_panel_3092855+2 | 563 | 94 | 0.1 | -3 |
| TSS_298204+2 | 562 | 94 | 0.1 | -3 |
| TSS_panel_4859935+1 | 562 | 95 | 0.1 | -5 |
| TSS_2565229+3 | 561 | 164 | 0.1 | -10 |
| TSS_panel_4854980+2 | 561 | 98 | 0.1 | -14 |
| TSS_3256658+2 | 558 | 16 | 0.1 | -12 |
| TSS_1188742+2 | 557 | 76 | 0.1 | -19 |
| TSS_949896+3 | 557 | 57 | 0.1 | -15 |
| TSS_386714+2 | 556 | 77 | 0.1 | -15 |
| TSS_308443+2 | 555 | 204 | 0.1 | -24 |
| TSS_3356616+2 | 554 | 95 | 0.1 | -17 |
| TSS_201116+3 | 552 | 28 | 0.1 | -15 |
| Gene          | Start | End   | Description          | Coordinate Type | Value |
|--------------|-------|-------|----------------------|-----------------|-------|
| TSS_1653983-3 | 90    | 28    | -14                 | 1               | 33    |
| TSS_11077951-2 | 25    | 0     | -16                 | 1               | 32    |
| TSS_3210012-3 | 18    | 23    | -1                  | 1               | 31    |
| TSS_1002801-3 | 90    | 16    | -13                 | 1               | 30    |
| TSS_1483061-3 | 90    | 10    | -17                 | 1               | 29    |
| TSS_2710002-3 | 48    | 16    | -25                 | 0               | 28    |
| TSS_306028-2  | 89    | 27    | -15                 | 1               | 27    |
| TSS_947457-2  | 89    | 27    | -15                 | 1               | 26    |
| TSS_126516-3  | 90    | 22    | -15                 | 1               | 25    |
| TSS_117898-5  | 89    | 13    | -16                 | 1               | 24    |
| TSS_2159819+2 | 89    | 18    | -17                 | 1               | 23    |
| TSS_3223384-2 | 89    | 18    | -16                 | 1               | 22    |
| TSS_3570219-2 | 89    | 24    | -17                 | 1               | 21    |
| TSS_3692875-2 | 89    | 29    | -15                 | 1               | 20    |
| TSS_1043437+2 | 89    | 13    | -17                 | 1               | 19    |
| TSS_2065369-2 | 89    | 19    | -16                 | 1               | 18    |
| TSS_2664909+2 | 89    | 21    | -16                 | 1               | 17    |
| TSS_900617-3  | 89    | 20    | -16                 | 1               | 16    |
| TSS_284078-2  | 88    | 11    | -15                 | 1               | 15    |
| TSS_1819360-3 | 88    | 8     | -16                 | 1               | 14    |
| TSS_1911058+5 | 88    | 19    | -17                 | 1               | 13    |
| TSS_1900763-2 | 88    | 14    | -13                 | 1               | 12    |
| TSS_2482704+2 | 86    | 32    | -3                  | 0               | 11    |
| TSS_10688876+2| 86    | 22    | -15                 | 1               | 10    |
| TSS_229066+2  | 85    | 15    | -20                 | 0               | 9     |
| TSS_65539+3   | 85    | 5     | -15                 | 1               | 8     |
| TSS_159687-3  | 85    | 30    | -16                 | 1               | 7     |
| TSS_401288+3  | 85    | 15    | -15                 | 1               | 6     |
| TSS_227445+3  | 84    | 4     | -20                 | 0               | 5     |
| TSS_2563332+2 | 84    | 4     | -7                  | 0              | 4      |
| TSS_332793+1  | 83    | 23    | -15                 | 1               | 3      |
| TSS_3757032+2 | 83    | 15    | -17                 | 1               | 2      |
| TSS_1783544+2 | 83    | 3     | -15                 | 1               | 1      |
| TSS_3230969-2 | 87    | 26    | -16                 | 1               | 31    |
| TSS_226873-4  | 87    | 27    | -1                  | 1               | 30    |
| TSS_3094766+2 | 87    | 11    | -16                 | 1               | 29    |
| TSS_1610668-2 | 87    | 5     | -15                 | 1               | 28    |
| TSS_2855591-2 | 87    | 31    | -6                  | 0               | 27    |
| TSS_4295659-2 | 87    | 6     | -1                  | 0               | 26    |
| TSS_149450+5  | 87    | 24    | -17                 | 1               | 25    |
| TSS_1163043+2 | 87    | 18    | -15                 | 1               | 24    |
| TSS_945899-3  | 87    | 15    | -15                 | 1               | 23    |
| TSS_54660+5   | 87    | 17    | -2                  | 0               | 22    |
| TSS_384277+2  | 87    | 20    | -13                 | 1               | 21    |
| TSS_1756516-3 | 87    | 21    | -15                 | 1               | 20    |
| TSS_1758310-3 | 87    | 15    | -15                 | 1               | 19    |
| TSS_2276870-3 | 87    | 22    | -15                 | 1               | 18    |
| TSS_151563+3  | 86    | 33    | -2                  | 0               | 17    |
| TSS_1431665-5 | 86    | 31    | -16                 | 1               | 16    |
| TSS_1154976+2 | 86    | 16    | -16                 | 1               | 15    |
| TSS_3495986+2 | 86    | 14    | -16                 | 1               | 14    |
| TSS_2349110-2 | 86    | 22    | -5                  | 0               | 13    |
| TSS_1548801+3 | 86    | 17    | -1                  | 1               | 12    |
| TSS_2009927+3 | 86    | 33    | 0.5                 | 16            | 11    |
| TSS_1312297+2 | 86    | 13    | -1                  | 1               | 10    |
| TSS_1363699-3 | 86    | 8     | -17                 | 1               | 9     |
| TSS_2210009-2 | 79    | 27    | -15                 | 1               | 8     |
| TSS_275501+2  | 79    | 27    | -15                 | 1               | 7     |
| TSS_358062+2  | 79    | 27    | -15                 | 1               | 6     |
| TSS_357217+2  | 79    | 27    | -15                 | 1               | 5     |
| TSS_2392248+2 | 79    | 27    | -15                 | 1               | 4     |
| TSS_2427690+2 | 79    | 27    | -15                 | 1               | 3     |
| TSS_151563-3  | 86    | 33    | -2                  | 0               | 2     |
| TSS_1410065-3 | 85    | 31    | -15                 | 1               | 1     |
| TSS_255343-2 | Op1896f_2 | AGTATAGTCC | 0,1 | TSS_242939+2 | Op0623f_1 | 9.28 |
| TSS_2421875+3 | EIP-SIG-2Comp | -143 | s | -15 | NA | 79 |
| TSS_26760+4 | TSS_271716-2 | Op1709r_2 | Rmet_1091 | 24 | -41 | NA | 0,1 | 9607 |
| TSS_975183+3 | 7.89 | 10606 | 3.08 | 0,1 | 0,1 | Rmet_2699 | 0 | 0,1 | 4,1 | Op0761f_1 | 82 |
| TSS_157899+4 | TSS_1535343+3 | -36 | 0 | 80 | NA | Op0518r_1 | -145 | no | -15 | 7.26 |
| TSS_2327982+2 | 80 |
| TSS_1576492-2 | 7 |
| TSS_2931327-2 | 9.28 | 7.51 | m | 230 | TSS_0927 | nuca | Op0267_3 | MET-EN-OxPhos |
| TSS_1638995-2 | 8 |
| TSS_2370444+2 | 11 |
| TSS_2931327-2 | 15 |
| TSS_1638995-2 | 1 |
| TSS_212515-4 | 5 |
| TSS_1012316+2 | 21 |
| TSS_041056+2 | 23 |
| TSS_1177246+2 | 8 |
| TSS_23421875+3 | 8 |
| TSS_3357391+2 | 18 |
| TSS_1770734+2 | 12 |
| TSS_2629570+3 | 13 |
| TSS_2447424+3 | 7 |
| TSS_255343+3 | 14 |
| TSS_2692477+2 | 8 |
| TSS_1587994-2 | 18 |
| TSS_2688218+5 | 18 |
| TSS_1536615+2 | 18 |
| TSS_2421875-3 | 24 |
| TSS_3636101-2 | 20 |
| TSS_2304431+3 | 8 |
| TSS_3195529-2 | 31 |
| TSS_7108+3 | 10 |
| TSS_2343897+3 | 10 |
| TSS_3558243-3 | 10 |
| TSS_3429768-2 | 18 |
| TSS_273023-3 | 14 |
| TSS_2278676-2 | 26 |
| TSS_1838250-2 | 4 |
| TSS_3178509-2 | 45 |
| TSS_1580998-3 | 16 |
| TSS_1710563-2 | 15 |
| TSS_1710563-2 | 15 |
| TSS_271716-2 | 18 |
| TSS_1938729-2 | 24 |
| TSS_2032227-2 | 17 |
| TSS_3225263-2 | 22 |
| TSS_2352557-2 | 9 |
| TSS_1085914-3 | 6 |
| TSS_2242298-2 | 23 |
| TSS_2242299-2 | 7 |
| TSS_0204101+2 | 23 |
| TSS_1127642+2 | 8 |
| TSS_2140102-2 | 21 |
| TSS_0892100+2 | 23 |
| TSS_2127487+3 | 8 |
| TSS_2931327-2 | 15 |
| TSS_0560812-2 | 25 |
| TSS_212515-4 | 5 |
| TSS_3001249+2 | 12 |
| TSS_975183+3 | 0 |
| TSS_1270165+2 | 9 |
| TSS_1270165+2 | 9 |
| TSS_2421875+3 | 8 |
| TSS_1177246+2 | 8 |
| TSS_2421875+3 | 8 |
| TSS_3357391+2 | 18 |
| TSS_1770734+2 | 12 |
| TSS_2629570+3 | 13 |
| TSS_2447424+3 | 7 |
| TSS_255343+3 | 14 |
| TSS_2692477+2 | 8 |
| TSS_1587994-2 | 18 |
| TSS_2688218+5 | 18 |
| TSS_1536615+2 | 18 |
| TSS_2421875-3 | 24 |
| TSS_3636101-2 | 20 |
| TSS_2304431+3 | 8 |
| TSS_3195529-2 | 31 |
| TSS_7108+3 | 10 |
| TSS_2343897+3 | 10 |
| TSS_3558243-3 | 10 |
| TSS_3429768-2 | 18 |
| TSS_273023-3 | 14 |
| TSS_2278676-2 | 26 |
| TSS_1838250-2 | 4 |
| TSS_3178509-2 | 45 |
| TSS_1580998-3 | 16 |
| TSS_1710563-2 | 15 |
| TSS_1710563-2 | 15 |
| TSS_271716-2 | 18 |
| TSS_1938729-2 | 24 |
| TSS_2032227-2 | 17 |
| TSS_3225263-2 | 22 |
| TSS_2352557-2 | 9 |
| TSS_1085914-3 | 6 |
| TSS_2242298-2 | 23 |
| TSS_2242299-2 | 7 |
| TSS_0204101+2 | 23 |
| TSS_1127642+2 | 8 |
| TSS_2421875+3 | 8 |
| TSS_3357391+2 | 18 |
| TSS_1770734+2 | 12 |
| TSS_2629570+3 | 13 |
| TSS_2447424+3 | 7 |
| TSS_255343+3 | 14 |
| TSS_2692477+2 | 8 |
| TSS_1587994-2 | 18 |
| TSS_2688218+5 | 18 |
| TSS_1536615+2 | 18 |
| TSS | GAACGACCTGCAGCAATCTGGAGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 6.38 | -18.60 | 0.00 | 880 | Rmet_6542 | 0 | Op20387_1 | NA |
| 3103 | TS53 | 2837546+2 | 53 | 19 | -48 | GCACACCTGCAGCAATCTGGAGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3105 | TS53 | 3696541+2 | 53 | 8 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 1 | Rmet_3221 | msd |
| 3105 | TS53 | 3696541+2 | 53 | 8 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 1510015+2 | 53 | 17 | 49 | GCACACCTGCAGCAATCTGGAGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 137200+2 | 53 | 29 | -49 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 1819476+3 | 53 | 8 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 1993934+3 | 53 | 7 | -49 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 2214324+3 | 53 | 8 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3101 | TS53 | 6649351+3 | 53 | 21 | -49 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 1365098+3 | 53 | 9 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 1652775+3 | 53 | 8 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 289165+3 | 53 | 8 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 1510035+2 | 53 | 8 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 1993934+3 | 53 | 7 | -49 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 1819476+3 | 53 | 8 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |
| 3103 | TS53 | 2214324+3 | 53 | 8 | -10 | TTGGACGCAACATCACTGCTGACGACAAAATTTTCTCATCACTGCT | 53 | 7 | 53 | -143 | -141 | 7.51 | 8.60 | 0.00 | 311 | Rmet_3223 | EIP-TRA-Pores |

**Note:** The table above contains data related to transcription start sites (TSS) and associated genomic sequences. The values correspond to various molecular and biochemical properties, including nucleotide sequences and distances. The data is presented in a tabular format with columns for TSS, sequence, and other relevant biological annotations.
| Gene     | Accession | Score | Description            | Start | End | Length |
|----------|-----------|-------|------------------------|-------|-----|--------|
| TSS_2500226+3 | TSS_3483384+2 | 6.56 | GATAAAATTC             | 4077  | 4073| 4 |
| ID   | Start | End   | Description |
|------|-------|-------|-------------|
| 4201 | 29    | 9     | TSS_49088-4 |
| 4202 | 9     | 29    | TSS_140350-5 |
| 4203 | 2     | 29    | TSS_326244-2 |
| 4204 | 2     | 29    | TSS_508141-2 |
| 4205 | 9     | 29    | TSS_674997-1 |
| 4206 | 9     | 29    | TSS_900313-2 |
| 4207 | 29    | 9     | TSS_156262-2 |
| 4208 | 9     | 29    | TSS_184309-1 |
| 4209 | 9     | 29    | TSS_262640-2 |
| 4210 | 29    | 9     | TSS_3030359-2 |
| 4211 | 2     | 9     | TSS_3424132 |
| 4212 | 9     | 27    | TSS_85737-2 |
| 4213 | 2     | 10    | TSS_185962-2 |
| 4214 | 29    | 11    | TSS_229498-2 |
| 4215 | 9     | 29    | TSS_236102-4 |
| 4216 | 9     | 9     | TSS_288988-2 |
| 4217 | 9     | 9     | TSS_345070-2 |
| 4218 | 9     | 14    | TSS_351971-2 |
| 4219 | 7     | 9     | TSS_351935-2 |
| 4220 | 3     | 29    | TSS_363766-2 |
| 4221 | 3     | 9     | TSS_363773-2 |
| 4222 | 10    | 9     | TSS_136127-2 |
| 4223 | 7     | 37    | TSS_2051406-3 |
| 4224 | 13    | 29    | TSS_218673-4 |
| 4225 | 5     | 9     | TSS_252169-8 |
| 4226 | 3     | 29    | TSS_53179-9 |
| 4227 | 10    | 9     | TSS_60303-7 |
| 4228 | 35    | 9     | TSS_115415-4 |
| 4229 | 11    | 9     | TSS_1291834-1 |
| 4230 | 12    | 9     | TSS_1455123-3 |
| 4231 | 12    | 9     | TSS_2155167-7 |
| 4232 | 12    | 9     | TSS_215529-11 |
| 4233 | 14    | 4     | TSS_2239114-4 |
| 4234 | 7     | 48    | TSS_34492-4 |
| 4235 | 3     | 36    | TSS_5069-3 |
| 4236 | 14    | 9     | TSS_1458238-2 |
| 4237 | 4     | 4     | TSS_1343450-2 |
| 4238 | 9     | 29    | TSS_219073-12 |
| 4239 | 14    | 3     | TSS_2298012-2 |
| 4240 | 9     | 35    | TSS_255573-22 |
| 4241 | 10    | 29    | TSS_3134362-2 |
| 4242 | 10    | 29    | TSS_3528285-2 |
| 4243 | 10    | 29    | TSS_69972-52 |
| 4244 | 9     | 9     | TSS_933647-2 |
| 4245 | 14    | 9     | TSS_1155378-2 |
| 4246 | 9     | 9     | TSS_126602-6 |
| 4247 | 9     | 9     | TSS_1418553-2 |
| 4248 | 9     | 9     | TSS_1810553-2 |
| 4249 | 9     | 9     | TSS_1891380-2 |
| 4250 | 2     | 9     | TSS_2526632-2 |
| 4251 | 2     | 9     | TSS_2526352-8 |
| 4252 | 2     | 9     | TSS_3507981-2 |
| 4253 | 2     | 9     | TSS_3099910-2 |
| 4254 | 2     | 9     | TSS_1098325-9 |
| 4255 | 2     | 9     | TSS_1898924-9 |
| 4256 | 7     | 9     | TSS_39467-3 |
| 4257 | 4     | 4     | TSS_308332-3 |
| 4258 | 4     | 4     | TSS_451373-3 |
| 4259 | 9     | 9     | TSS_1092457-3 |
| 4260 | 3     | 3     | TSS_9976-4 |
| Gene/Motif       | Start | End   | Rank | Score | Description          |
|-----------------|-------|-------|------|-------|----------------------|
| TSS_1889760+2   | 844   | 848   | 21   | 5     | 3.64 ± 1.8          |
| TSS_2744260+2   | 662   | 667   | 4    | 1.88  | 6.64 ± 3.5          |
| TSS_313501+2    | 6     | 11    | 4    | 3.60  | 5.91 ± 7.9          |
| TSS_3436997+1   | 223   | 230   | 6    | 1.88  | 1.56 ± 3.9          |
| TSS_371042+7    | 5     | 11    | 6    | 1.88  | 1.56 ± 3.9          |
| TSS_3814044+2   | 5     | 10    | 7    | 1.88  | 1.56 ± 3.9          |
| TSS_393535+2    | 5     | 11    | 7    | 1.88  | 1.56 ± 3.9          |
| TSS_303878+2    | 5     | 10    | 8    | 1.88  | 1.56 ± 3.9          |
| TSS_305022+1    | 7     | 12    | 9    | 1.88  | 1.56 ± 3.9          |
| TSS_349107+2    | 4     | 9    | 10   | 1.88  | 1.56 ± 3.9          |
| TSS_308789+2    | 5     | 10    | 13   | 1.88  | 1.56 ± 3.9          |
| TSS_3227553-1   | 7     | 12    | 17   | 1.88  | 1.56 ± 3.9          |
| TSS_1188565+3   | 1     | 5     | 21   | 1.88  | 1.56 ± 3.9          |
| TSS_2453031+3   | 3     | 8     | 23   | 1.88  | 1.56 ± 3.9          |
| TSS_219167+4    | 6     | 11    | 26   | 1.88  | 1.56 ± 3.9          |
| TSS_4399+5      | 4     | 9     | 30   | 1.88  | 1.56 ± 3.9          |
| TSS_195608+3    | 1     | 5     | 33   | 1.88  | 1.56 ± 3.9          |
| TSS_140478+2    | 5     | 10    | 37   | 1.88  | 1.56 ± 3.9          |
| TSS_123222+3    | 5     | 10    | 41   | 1.88  | 1.56 ± 3.9          |
| TSS_3227553-1   | 7     | 12    | 45   | 1.88  | 1.56 ± 3.9          |
| TSS_1783615+3   | 2     | 7     | 49   | 1.88  | 1.56 ± 3.9          |
| TSS_2358384+2   | 4     | 9     | 53   | 1.88  | 1.56 ± 3.9          |
| TSS_2782247+2   | 2     | 7     | 57   | 1.88  | 1.56 ± 3.9          |
| TSS_283428+2    | 2     | 7     | 61   | 1.88  | 1.56 ± 3.9          |
| TSS_281434+2    | 2     | 7     | 65   | 1.88  | 1.56 ± 3.9          |
| TSS_689426+2    | 2     | 7     | 69   | 1.88  | 1.56 ± 3.9          |
| TSS_1580346-2   | 4     | 9     | 73   | 1.88  | 1.56 ± 3.9          |
| TSS_1871776+2   | 3     | 8     | 77   | 1.88  | 1.56 ± 3.9          |
| TSS_2159446+2   | 7     | 12    | 81   | 1.88  | 1.56 ± 3.9          |
| TSS_2405721+2   | 7     | 12    | 85   | 1.88  | 1.56 ± 3.9          |
| TSS_2891177+2   | 5     | 10    | 89   | 1.88  | 1.56 ± 3.9          |
| TSS_3304532-2   | 5     | 10    | 93   | 1.88  | 1.56 ± 3.9          |
| TSS_3417412+2   | 5     | 10    | 97   | 1.88  | 1.56 ± 3.9          |
| TSS_3050200+2   | 1     | 6     | 101  | 1.88  | 1.56 ± 3.9          |
| TSS_3556315+2   | 6     | 11    | 105  | 1.88  | 1.56 ± 3.9          |
| TSS_3571020+2   | 6     | 11    | 109  | 1.88  | 1.56 ± 3.9          |
| TSS_3591082+2   | 6     | 11    | 113  | 1.88  | 1.56 ± 3.9          |
| TSS_3615319+2   | 6     | 11    | 117  | 1.88  | 1.56 ± 3.9          |
| TSS_3616802+2   | 4     | 9     | 121  | 1.88  | 1.56 ± 3.9          |
| TSS_3814032+2   | 3     | 8     | 125  | 1.88  | 1.56 ± 3.9          |
| TSS_3717810+2   | 4     | 9     | 129  | 1.88  | 1.56 ± 3.9          |
| TSS_3838310+2   | 3     | 8     | 133  | 1.88  | 1.56 ± 3.9          |
