Table S1. Oligodeoxynucleotides used in this work: Probes for Northern blot analysis and primers for cloning, site directed mutagenesis and qRT-PCR

Name	Sequence	Purpose
Probes for Northern Blot		
p-0680a	5'-CGTCGCCGCTGCTGCTACAGGTC-3'	24 nt probe for CcsR1 (1)
pCcsR1 (73 nt)	5'-AAGGACGGCGACACCAGGGAGGAGGAGAGGCGTCGCCGCTGCTGCTACAGGTCCCGGGAGGAGGTGGGACCTC-3	83 nt probe for CcsR1 for differential detection of CcsR1-3, based on sequence determined in (1)
pCcsR2 (80 nt)	5,-TGGTGCAGCGACCCCAGGGAGGAGGAGGGGGCCGCTGCGGTTCCGATCGGCCAGGGAGGAGGAGAGGCCGTTCGAAGAGG-3	80 nt probe for CcsR2 for differential detection of CcsR1-3 based on sequence determined in (1)
pCcsR3 (88 nt)	5'-GGGGGCGACGGCTCCAGGGAGGAGGAGGGAGCCGCCGCTTGGTCATCGGCACCGCGGGAGGAGGTGCAGTGCCGAAGTCCAGTGGGCA-3	88 nt probe for CcsR3 for differential detection of CcsR1-3 based on sequence determined in (1)
p-1543	5'-CAGGGAGGTATGAAGCGGACGAG-3'	Probe for RSs1543
p-5S	5'-CTTGAGACGCAGTACCATTG-3'	Probe for 5S rRNA (1)
Primer for Cloning		
RSP_4352_5'_HindIII_fw	5'-AAGCTTCAGGTCGGGCGAAAGG-3'	Cloning of 16S rRNA (RSP_4352 Promoter) into pPHU235, forward primer
RSP_4352_5'_BamHI_rv	5'-GGATCCGTTTCTAGGAGCAGACGGCCC-3'	Cloning of 16S rRNA (RSP_4352 Promoter) into pPHU235, reverse primer
RSP_6037_for_BamHI	5'-GGATCCAACAGCGCGACGGCAAAG-3	Cloning of RSP_6037 + CcsR1-4 into pRK4352, forward primer
CcsR1_for_BamHI	5'-GGATCCTTTCCTGCGAGGTCCCAC-3'	Cloning of CcsR1, CcsR1+2, CcsR1-3 and CcsR1-4 into pRK4352/pBBR4352, forward primer
CcsR1-rev-EcoRI	5'- GAATTCAAAGGACGGCGACACCAG -3	Cloning of CcsR1 into pRK4352, reverse primer
CcsR2-for-BamHI	5'-GGATCCTCTTCGAACGGCCTCTCC-3'	Cloning of CcsR2 into pRK4352, forward primer
CcsR2-rev-EcoRI	5'-GAATTCATCTGGTGCAGCGACCC -3'	Cloning of CcsR12 and CcsR1+2 into pRK4352, reverse primer
CcsR3-rev-EcoRI	5'-GAATTCGGGGGCGACGGCTCC-3'	Cloning of CcsR1 -3 into pRK4352, reverse primer
CcsR4_rev_XbaI	5'-TCTAGACTCATCGGCCGCATGAAT -3'	Cloning of CcsR1-4 into pBBR4352, reverse primer
CcsR4_rev_EcoRI	5'-GAATTCCTCATCGGCCGCATGAATC-3'	Cloning of RSP_6037 + CcsR1-4 /CcsR1-4 into pRK4352, reverse primer
up2591Kpn-f	5'-GGTACCCTGGGCCCGGCGATGCATC-3'	Cloning of *flhR* upstream fragment, forward primer
up2591Pst-r	5'-CTGCAGCTCTTCCTCCCGTTTCCGCGC-3'	Cloning of *flhR* upstream fragment, reverse primer
down2591Pst-f	5'-CTGCAGGGAGCGTCGCGTGACGACAGA-3	Cloning of *flhR* downstream fragment, forward primer
down2591Hind-r	5'-AAGCTTCATCCGGCTCCCCGAGGGGGT-3'	Cloning of *flhR* downstream fragment, reverse primer
RSP_2591_Xba_fw	5'-TCTAGACGGCAACATTTGCCCGCC-3'	Cloning 166 bp 5'-UTR and 1st 18 bases of RSP_2591 into pPHU16S for lacZ fusion, forward primer
RSP_2591_Pst_rv	5'-CTGCAGTCCCGAAACATGTCCCATC-3'	Cloning 166bp 5'-UTR and 1st 18 bases of RSP_2591 into pPHU16S for lacZ fusion, reverse primer
RSP_2876_Xba_fw	5'-TCTAGAACCATTCCTGCGTCAC-3'	Cloning 204 bp in 5' direction from ATG and 1st 18 bases of RSP_2876 into pPHU16S for lacZ fusion, forward primer
RSP_2876_Pst_rv	5'-CTGCAGCAGGTCGAAATTGT-3'	Cloning 204 bp in 5' direction from ATG and 1st 18 bases of RSP_2876 into pPHU16S for lacZ fusion, reverse primer
RSP_2877_XbaI_fw	5'-TCTAGATCACCACGGCCTCCA-3'	Cloning 233 bp in 5' direction from ATG and 1st 18 bases of RSP_2877 into pPHU16S for lacZ fusion, forward primer
RSP_2877_PstI_rv	5'-CTGCAGGCCTCCGTCCTT-3'	Cloning 233 bp in 5' direction from ATG and 1st 18 bases of RSP_2877 into pPHU16S for lacZ fusion, reverse primer
RSP_4050_Xba_fw	5'-TCTAGATCTCGAGAAGCACGCG-3'	Cloning first 69 bp of RSP_4050 (pdhB) and 80 bp in 5' direction from ATG into pPHU16S for lacZ fusion, forward primer
RSP_4050_Pst_rv	5'-CTGCAGCAGCCATTTCGCGA-3'	Cloning first 69 bp of RSP_4050 (pdhB) and 80 bp in 5' direction from ATG into pPHU16S for lacZ fusion, reverse primer
RSP_6132_Xba_fw	5'-TCTAGACCGCCATCCGCCCTC-3'	Cloning 180 bp 5'-UTR and 1st 18 bases of RSP_6132 into pPHU16S for lacZ fusion, forward primer
RSP_6132_Pst_rv	5'-CTGCAGCGGGGTCTTCCAGG-3'	Cloning 180 bp 5'-UTR and 1st 18 bases of RSP_6132 into pPHU16S for lacZ fusion, reverse primer
2591ovfbam1	5'-GGATCCTTGCCCGCCGAAGGGAAC-3	Cloning of *flhR* into pRK4352, forward primer
2591ovRkpn1	5'-GGTACCCCGCGCGAAGATCCGCG-3'	Cloning of *flhR* into pRK4352, reverse primer
Mut2591GGA27CCTfw	5'-GGGAGGACCTAGCCCGCGCGGAAACGGG-3	Base exchange in RSP_2591 GGA27 to CCT27 for analysis in pPHU16S, forward primer
Mut2591GGA27CCTrv	5'-CGGGCTAGGtCCTCCCTGCGCGGTCTTC-3'	Base exchange in RSP_2591 GGA27 to CCT27 for analysis in pPHU16S, reverse primer
Primer for generation of templates for T7-RNA polymerase		
T7_CcsR1_f	5'-TAATACGACTCACTATAGAGGTCCCACCTCCTCCTC-3'	Amplification of a DNA fragment consiting of a T7 promoter and the 83 bp stretch encoding CcsR1 based on (1), forward primer
T7_CcsR1_r	5'-AAGGACGGCGACACCAGGG-3'	Amplification of a DNA fragment consiting of a T7 promoter and the 83 bp stretch encoding CcsR1 based on (1), reverse primer
T7_PcrZ_f	5'-TAATACGACTCACTATAGGGCACGCCGGAGTGGTAAC-3'	Amplification of a DNA fragment consiting of a T7 promoter and ta 135 bp stretch encoding PcrZ , forward primer
T7_PcrZ_r	5'-GCAGCGAAGGATGCCCGACAG-3'	Amplification of a DNA fragment consiting of a T7 promoter and ta 135 bp stretch encoding PcrZ , reverse primer
T7_2591_f	5'-TAATACGACTCACTATAGGTTGCCCGCCGAAGGGAAC-3'	Amplification of a DNA fragment consiting of a T7 promoter and a 177 bp stretch encoding the putative CcsR1 binding site in the flhR 5'UTR, forward primer
T7_2591_r	5'-TCCCGAAACATGTCCCATC-3'	Amplification of a DNA fragment consiting of a T7 promoter and a 177 bp stretch encoding the putative CcsR1 binding site in the flhR 5'UTR, reverse primer
T7_0285_f	5'-TAATACGACTCACTATAGCCCGCCTCGACATGGAG-3'	Amplification of a DNA fragment consiting of a T7 promoterand a 130 bp stretch encoding a putative PcrZ binding site for bchN, forward primer
T7_0285_r	5'-GATGATGCCGGTCAGTCCGC-3'	Amplification of a DNA fragment consiting of a T7 promoterand a 130 bp stretch encoding a putative PcrZ binding site for bchN, reverse primer
Primer for qRT-PCR		
RSP2576up	5'-GGTCATGGTCGAGATCAAGG-3'	Forward primer for qRT-PCR RSP_2576
RSP2576down	5'-AGGTTCGTCTTCTGGCTGAG-3'	Reverse primer for qRT-PCR RSP_2576
2580RT-F	5'-GATCCGCGCTCTCCTGC-3'	Forward primer for qRT-PCR RSP_2580
2580RT-R	5'-CAGGGGCGAATTCGCCG-3'	Reverse primer for qRT-PCR RSP_2580
2579RT-F	5'-CATCCGCTCGTTCAATGC-3'	Forward primer for qRT-PCR RSP_2579
2579RT-R	5'-CAGACATGGCACTCCGAC-3'	Reverse primer for qRT-PCR RSP_2579
2877_real-A	5'-TACGAGCAGGCCAAGGAT-3'	Forward primer for qRT-PCR RSP_2877
2877_real-B	5'-GGCGGTTGTTCACCAGTT-3'	Reverse primer for qRT-PCR RSP_2877
RSP4050_real_Up	5'-AAGCTCGCACCGCACGAATA-3'	Forward primer for qRT-PCR RSP_4050
RSP4050_real_ Dwn	5'-ATCATGGTGGCCGTCGTGAT-3'	Reverse primer for qRT-PCR RSP_4050
6132RT-F	5'-GGAAGACCCCGCGTTAC -3'	Forward primer for qRT-PCR RSP_6132
6132RT-R	5'- CTTGCGCTTGGCGCAGG-3'	Reverse primer for qRT-PCR RSP_6132

Table S2. Plasmids used in this work

Plasmid	Description	Source
pRK415	broad-host-range cloning vector, Tc^r	see (2)
pRK4352	pRK415 with the RSP_4352 16S rRNA promoter, Tc^r	see (3)
pRCcsR1	pRK4352 with the sRNA Ccsr1 under control of the RSP_4352 16S rRNA promoter, Tc^r	This study
pRCcsR2	pRK4352 with the sRNA Ccsr2 under control of the RSP_4352 16S rRNA promoter, Tc^r	This study
pRCcsR1+2	pRK4352 with the 2 sRNAs Ccsr1+2 under control of the RSP_4352 16S rRNA promoter, Tc^r	This study
pRCcsR1-3	pRK4352 with the 3 sRNAs Ccsr1-3 under control of the RSP_4352 16S rRNA promoter, Tc^r	This study
pRCcsR1-4	pRK4352 with the 4 sRNAs Ccsr1-4 under control of the RSP_4352 16S rRNA promoter, Tc^r	This study
pR6037_CcsR1-4	pRK4352 with RSP_6037 and the 4 sRNAs Ccsr1-4 under control of the RSP_4352 16S rRNA promoter, Tc^r	This study
pRK_*flhR*	pRK4352 with RSP_2591 under control of the RSP_4352 16S rRNA promoter, Tc^r	This study
pBBR4352	pBBR1MCS2 with the RSP_4352 16S rRNA promoter, Km^r	see (3)
pBCcsR1-4	pBBR4352 with the 4 sRNAs Ccsr1-4 under control of the RSP_4352 16S rRNA promoter, Km^r	This study
pPHU281	Tc^r	see (5)
pUC4K	Km^r	
pPHU2591up-KM-down	pPHU281 with RSP_2591 (*flhR*) upstream and downstream fragments flanking the Km-resistance cassette from pUC4K, Tc^r	this study
pPHU235	broad-host-range *lacZ*-fusion vector, Tc^r	see (6)
pPHU16S	pPHU235 with the RSP_4352 16S rRNA promoter, Tc^r	This study
pPHU16S2591	pPHU16S with the first 18bp of RSP_2591 (*flhR*/*afdR*) and 166 bp of the 5' region with a putative CcsR1-4 binding site in a lacZ fusion under control of the 16S rRNA promoter	This study
$pPHU16S2591_{mut3}$	pPHU16S with the first 18bp of RSP_2591 (*flhR*/*afdR*) and 166 bp of the 5' region with a putative CcsR1-4 binding site in a lacZ fusion under control of the 16S rRNA promoter, triple mutation in the CcsR1-4 interaction site	This study
pPHU16S2876	pPHU16S with the last 194 bp of RSP_2877 with parts of a putative CcsR1 binding site, the 10bp UTR between RSP_2877 and RSP_2876 and the first 18 bp of RSP_2876 in a lacZ fusion under control of the RSP_4352 16S rRNA promoter	This study
pPHU16S2877	pPHU16S with the last 205 bp of RSP_2878, the 28 bp UTR between RSP_2878 and RSP_2877 including a putative CcsR1 binding site and the first 18bp of RSP_2877 (coxL) in a lacZ fusion under control of the RSP_4352 16S rRNA promoter	This study
pPHU16S4050	pPHU16S with the first 69 bp of RSP_4050 (pdhB) including a putative CcaR1-4 binding site and 80 bp of the respective 5' region in a lacZ fusion under control of the RSP_4352 16S rRNA promoter	This study
pPHU16S6132	pPHU16S with the first 18 bp of RSP_6132(pqqA) and 180 bp of the 5' region with a putative CcaR1-4 binding site in a lacZ fusion under control of the 16S rRNA promoter	This study
pPHU16S*bchN*	pPHU4352 with the first 60 bp of *bchN* (RSP_0285) and 105 bp of the 5'region with a putative binding site for the sRNA PcrZ in a lacZ fursion under control of the 16S rRNA promoter	see (3)
pDrive cloning vector	Ap^r, Km^r	Quiagen
pJet1.2 cloning vector	Ap^r	Thermo Scientific

Table S3. Bacterial strains used in this work

Strain	Description	Antibiotics (µg ml^{-1})	Source
E. coli			
E. coli JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi (lac–proAB)*	-	New England Biolabs
E. coli S17-1	*recA pro hsdR RP4-2-Tc::Mu-Km::Tn7*	-	See (7)
R. sphaeroides			
R. sphaeroides 2.4.1	Wild type	-	See (8)
R. sphaeroides 2.4.1 Δ*hfq*	*hfq* mutation in wild type 2.4.1, Sp^r	Spectinomycin (10)	See (9)
R. sphaeroides 2.4.1 Δ*flhR*	*flhR* mutation in wild type 2.4.1, Km^r	Kanamycin (25)	This study

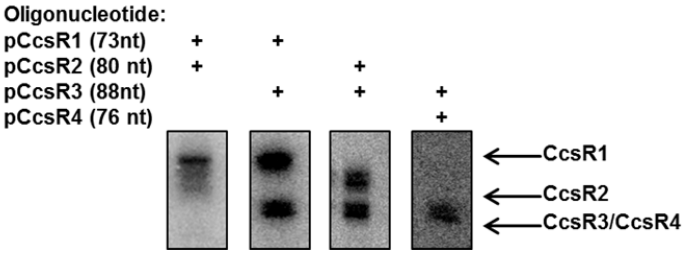

Figure S1:Differential detection of CcsR1-3/4. Depicted are data from Northern blot experiments of *R. sphaeroides* 2.4.1 under heat stress conditions. CcsR1-4 were detected using specific probes for the individual sRNAs. Specific detection of CcsR1 and 2 was possible while CcsR3+4 showed cross hybridization of their respective probes.

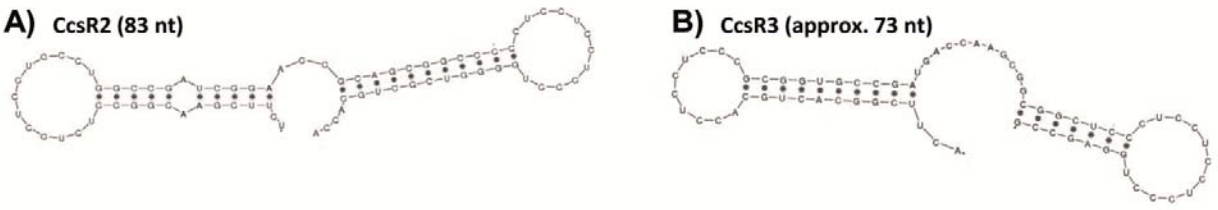

Figure S2. Predicted secondary structures of CcsR2 and CcsR3: A)+B) Displayed are minimum free energy (MFE) structures of CcsR2+3. Dots in the base pairing regions indicate that the predicted base interactions occur in the MFE structures as well as in the predicted centroid structures. All secondary structures depicted in this figure were created using Sfold (9).

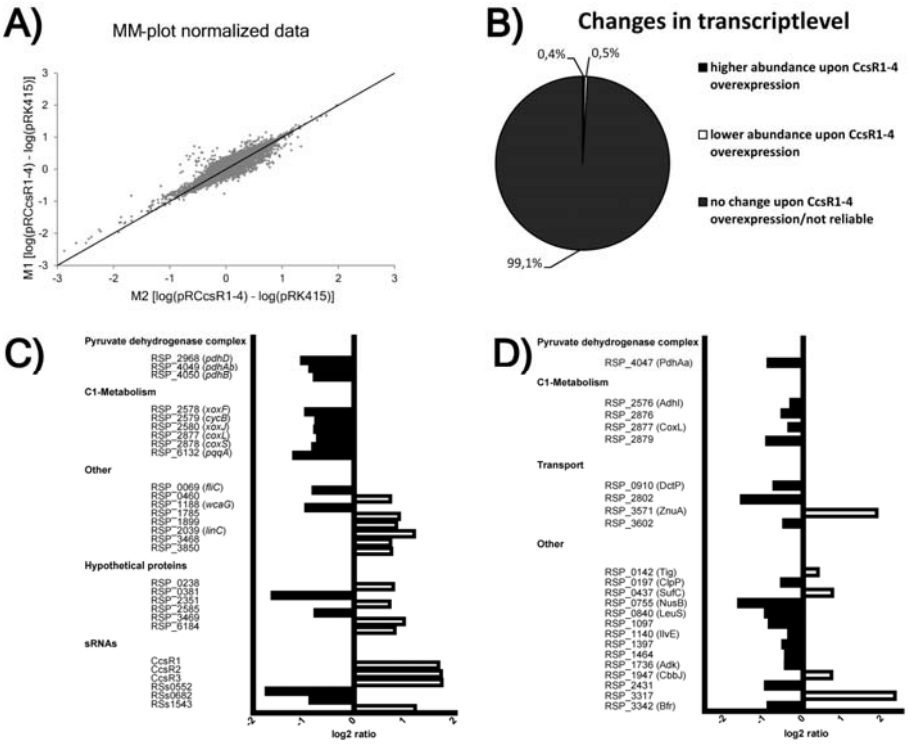

Figure S3. Global analysis of the transciptome data of *R. sphaeroides* 2.4.1 (pRCcsR1-4) vs. *R. sphaeroides* 2.4.1 (pRK415). Depicted are data from a microarray analysis and a gel based proteome analysis comparing *R. sphaeroides* (pRCcsr1-4) to the control strain *R. sphaeroides* (pRK415) under aerobic conditions in early exponential growth phase. Two individual microarrays (biological replicates) were hybridized to two different pools of RNAs from three independent experiments for each strain. The data of the microarray analysis have been deposited in NCBI's Gene Expression Omnibus (10) and are accessible through GEO Series accession number XY (will be provided in the final manuscript).A) MM-plot comparing the log ratios between the two replicates. Displayed is the log ratio of biological replicate one (M1) against the log ratio of technical replicate two (M2) B) 0.4% of the transcripts with reliable A-Value showed higher abundance (log_2 (ratio) ≥ 0.7) in *R. sphaeroides* (pRCcsr1-4) in comparison to the control strain, while 0.5% of the transcripts showed lower abundance (log_2 (ratio) ≤-0.7). C) Genes with changed RNA abundance in the microarray D) Genes with changed protein abundance in the proteome analysis(log_2 ratio ≤-0,3; p≤0.05 or log_2 ratio ≥0,3; p≤0.05)

Table S4. Whole genome sRNA-mRNA interaction predictions for CcsR1-4: Whole genome sRNA-mRNA interaction predictions were employed for CcsR1-4 with help of INTARNA (11). In order to increase the reliability of the prediction, a whole genome interaction prediction for CcsR1 by RNAPREDATOR was conducted (12). The data show sRNA-mRNA interaction predictions that indicate a redundant function of three or 4 of the sRNAs on the same messenger RNA. For these redundant interaction predictions high amounts of free energy are typical. Also these interactions are predicted by INTARNA as well as by RNAPREDATOR. On the other hand there are predicted interactions that indicate unique target mRNAs for CcsR1. Predicted interaction partners of CcsR1-4 that were tested in the *in vivo* reporter system are highlighted in bold.

			IntaRNA				RNApredator
			CcsR1	CcsR2	CcsR3	CcsR4	CcsR1
Gene	Name	Function	Energy [kcal/mol]	Energy [kcal/mol]	Energy [kcal/mol]	Energy [kcal/mol]	Energy [kcal/mol]
Redundant hits							
RSP_6040		Hypothetical protein	-27.4	-27.4	-25.6	-25.6	-22.70
RSP_2255		AMP-binding domain protein	-22.7	-22.7	-21.9	-21.9	
RSP_1576	*trxB*	Thioredoxin reductase	-22	-22.6	-21.2	-21.3	-19.59
RSP_2591	***flhR***	**Two component LuxR family transcriptional_regulator**	**-21.8**	**-24.3**	**-22.4**	**-22.4**	**-20.26**
RSP_1195	*comF*	Competence protein F	-21.7	-18.8	-23.3	-18.6	
RSP_2749		P4 family integrase	-21.5	-23.1	-27	-23.1	
RSP_2872	*aglF*	ABC alpha-glucoside transporter, inner membrane subunit AglF	-21.4	-22.7	-23.8	-23.8	-20.17
RSP_0573	*phoB*	Response regulator receiver protein	-21.2	-21.2	-19.8	-19.8	-19.86
RSP_2844		putative GTP-binding protein	-20.9	-20.9	-20.8	-20.8	-16.81
RSP_2365		ABC sugar (ribose) transporter, periplasmic substrate-binding subunit	-20.3	-20.3	-18.9	-18.9	-19.67
RSP_2669		ABC sugar (glycerol) transporter, ATPase subunit	-19.7	-17.3	-17.3	-17.3	
RSP_6132	***pqqA***	**Coenzyme PQQ synthesis protein PqqA**	**-19.7**	**-19.7**	**-18.4**	**-18.4**	**-17.96**
RSP_2104		Hypothetical protein	-19.3	-21.1	-21.1	-21.1	
RSP_0793	*pqqB*	Pyrroloquinoline quinone biosynthesis protein PqqB	-19.1	-19.1	-18.3	-18.3	-19.26
RSP_0488	*uxaC*	Glucuronate isomerase	-19	-19.7	-19.7	-19.7	-18.55
RSP_0725		thioredoxin, thioldisulfide interchange protein	-19	-19	-18.8	-18.8	
RSP_2939		Condensin subunit ScpB	-18.8	-18.8	-17.9	-17.9	
RSP_4050	***pdhB***	**Branched-chain alpha-keto acid dehydrogenase subunit E2**	**-18.2**	**-18.2**	**-17.3**	**-17.3**	
CcsR1 specific							
RSP_2719		AsnC family transcriptional regulator	-20.3				
RSP_0598	*dut*	deoxyuridine 5'-triphosphate nucleotidohydrolase	-19.6				
RSP_2876		**Putative carbon monoxide dehydrogenase medium chain**	**-18.4**				
RSP_2521	*nuoG*	NADH dehydrogenase subunit G	-18.3				
RSP_0487		TRAP dicarboxylate family transporter DctP subunit	-17.1				
RSP_1438		Iron-hydroxamate transporter permease subunit	-17				
CcsR2 specific							
RSP_2889		Transcriptional regulator		-22.2			
RSP_2376	*kbl*	2-amino-3-ketobutyrate coenzyme A ligase		-19.9			
RSP_2003	*yibQ*	YibQ protein		-19			
RSP_2084		Hypothetical protein		-18.7			
RSP_2526	*nuoK*	NADH dehydrogenase subunit K		-17.8			
RSP_1019		Putative glycolate oxidase subunit protein		-17.7			
RSP_2381		3-methyladenine DNA glycosylase		-17.2			
CcsR3 specific							
RSP_0640		Putative_N-methylhydantoinase A			-24		
RSP_1077		LysR family transcriptional regulator			-19		
RSP_6242		Hypothetical protein			-18.3		
RSP_2480		Hypothetical protein			-17.3		
CcsR4 specific						-16.7	
RSP_2220	*fliG*	Flagellar motor switch protein				-16.5	
RSP_2932	*hutC*	GntR family transcriptional regulator				-16,5	

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