

Materials and Methods

Strains and culture conditions

C. thermocellum wild-type (WT) strains ATCC 27405 DSM 1313 were obtained from their respective culture collections. *C. thermocellum* ethanol adapted (EA) mutant derived from strain ATCC 27405 has been described previously (1). *C. thermocellum* strain DSM 1313 was cultured in modified M122 medium (2, 3) at 55°C and all *C. thermocellum* growth studies contained cellobiose as the sole carbon source. *Saccharomyces cerevisiae* InvSc1 was grown at 30°C in YPD medium prior to transformation and SD-ura medium upon transformation with URA3+ plasmids, and *E. coli* Top10 was cultured in LB medium at 37°C. To select for the presence of plasmids, thiamphenicol (10 mg/ml) and chloramphenicol (12 mg/ml) were used for *C. thermocellum* and *Escherichia coli*, respectively.

Resequencing

Resequencing was conducted essentially as described previously (4). Briefly, genomic DNA from *C. thermocellum* wild-type ATCC27405 or EA mutant was isolated using a Wizard Genomic DNA purification kit (Promega, Madison, WI). Genomic DNA of wild-type ATCC 27405 and EA mutant was sent to NimbleGen facility for CGS service following the company's procedure. Pyrosequencing using the Roche 454 GS FLX System (454 Life Sciences, Bradford, CT) was carried out using both shotgun and paired-end DNA library preparation methods and have been deposited in the National Center for Biotechnology Information (NCBI) short-read archive database (SRX030163.2 and SRX030164.1, respectively). The GSMapper application in the 454 GS FLX software package 1.1.03 (454 Life Sciences) was used to map the reads generated from GS FLX onto the *C. thermocellum* ATCC 27405 reference genome (GenBank accession: CP000568).

Analysis of distribution of mutations inferred from pyrosequencing data

Distribution of the mutations across the genome was analyzed by calculating quantities of each type of mutation (total mutations, non-synonymous, synonymous, indels, and number of mutations in the intergenic regions)

within each 1000 and each 5000 nucleotides across the genome. For each position in the genome (L_i) we also calculated the Local Mutation Frequency (LMP) as $LMP = 1 / ((M_{up} - L_i) + (L_i - M_{down})) / 2 * 100$, where L_{up} is the start position of the closest upstream mutation and L_{down} is the start position of the closest downstream mutation. Mutation hot spots were identified by manual curation of the distributions and by analysis of potential functional relationships between genes comprising each hot spot. In bacteria the neighboring gene or genes may comprise an operon and are likely involved in the same biological process or metabolic pathway. To find out if mutations target such functionally related genes we calculated the number of mutations in each pair of genes and in genes that belong to an operon with subsequent manual analysis of the effected protein products and their annotation with MetaCyc pathways (5) using *C. thermocellum* pathway genome database available in the BioEnergy Science Center (BESC) Knowledgebase (http://cricket.ornl.gov/cgi-bin/beocyc_home.cgi). At least one non-synonymous mutation in two adjacent genes or in a gene of the operon was required to consider mutations as functionally related. The intergenic region was considered as functionally related to a hot spot if the distance from a mutation in the region to a mutated gene/operon was less than 400 nucleotides.

Plasmid and strain construction

Yeast gap repair cloning (6) was used to insert the WT and EA *adhE* alleles into plasmid pAMG205 (2), deleting *pyrF* and creating an artificial operon with the antibiotic resistance gene, *cat*, for expression in *C. thermocellum*. Briefly, the *adhE* alleles were PCR amplified from *C. thermocellum* WT and EA using primers **GGCCTAATGACTGGCTTTTATAATAAAGGAGGTCGACGTCATGACGAAAATAGCGAATAAATAC** and **G** and **CAGTCTTCCGACTGAGCCTTTTGTTTTCTCGAGGCCCGGGTCATTTCTTCGCACCTCCGTAAT** (priming regions in **bold**). These PCR products were each transformed along with *ZraI*+*SmaI* digested pAMG205 into *S. cerevisiae* InvSc1 (Invitrogen, Carlsbad, CA) via a modified Lazy Bones protocol (7). Plasmid was isolated using a Zymoprep Yeast Plasmid Miniprep II kit (Zymo, Orange, CA, USA) and electroporated into *E. coli* Top10 for verification, resulting in pAMG242 (mutant *adhE*) and pAMG249 (WT *adhE*). Empty vector control plasmid pAMG226 (pAMG205 \square *pyrF*) was constructed via restriction digestion of

pAMG205 with *ZraI* and *SmaI*, followed by self-ligation of the 6.9 kb fragment and transformation into *E. coli* Top10. Plasmids were then transformed into *C. thermocellum* DSM 1313 via electroporation as described (2) with modifications. To make *C. thermocellum* electrocompetent, 500 ml cultures were grown to an OD₆₀₀ of 0.6 - 1.0 and placed on ice to cool. Cells were then centrifuged at 6000 × g for 15 minutes at room temperature in a Beckman Coulter Avanti J-25 centrifuge with a JA-10 rotor, the supernatant was removed, and the cell pellet was washed twice with electroporation buffer (250 mM sucrose, 10% glycerol, 100 μM MOPS pH 7.0, 0.5 mM MgCl₂, 0.5 mM MgSO₄) in a similar fashion. Cells were then brought into a Coy anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) and resuspended in 500 ml electroporation buffer. Plasmid DNA (ca. 500 ng) was mixed with 20 ml washed cells in a pre-chilled 1 mm electroporation cuvette, and the cell/DNA mixture was subjected to a 1.2 kV, 1.5 msec square wave pulse using a BioRad GenePulser XCell. Electroporated cells were resuspended in 1 ml growth medium and mixed with ca. 25 ml growth medium supplemented with 0.8% agar and thiamphenicol. Plates were allowed to solidify and were placed in 2.5 L AnaeroPack Rectangular Jars (bioMerieux, Durham, NC, USA) and incubated at 51°C for up to one week. Colonies were picked into liquid growth medium containing thiamphenicol stored at -80°C for preservation.

***C. thermocellum* growth experiments**

C. thermocellum strains carrying pAMG226 (empty vector), pAMG249 (WT *adhE*), and pAMG242 (mutant *adhE*) were grown to an OD₆₀₀ of 1.0, and used as inocula (2% v/v) into 25 ml sealed Balch tubes containing 10 ml medium supplemented with 0, 1, 2, 3, and 5% (v/v) added ethanol. Cultures were incubated at 55°C, and growth was monitored via absorbance using a ThermoSpectric Genesys 10 vis spectrophotometer at 600 nm.

ADH enzyme assays

The ADH enzyme assays are based on previously described methods (8, 9). Briefly, half liter cultures of each strain were grown to OD₆₀₀ = 0.6 and centrifuged at 4°C at 6000 x g in a Beckman Coulter Avanti J-25 centrifuge with a JA-10 rotor. The culture was brought into the anaerobic chamber, and the supernatant was

removed. All further steps were carried out in the anaerobic chamber. The cell pellet was resuspended in 4 mL 100 mM Tris-HCl (pH 7.6) 0.1 mM dithiothreitol buffer, transferred to a 10 mL glass beaker, and sonicated for 8 minutes with 10 second pulses and 10 second pauses at 50% of the max intensity using a Misonix Sonicator 4000 with a microtip. Crude cell extract was centrifuged at 14000 x g for 25 min and stored on ice until assayed. The anaerobic reaction mixture contained 100 mM Tris-HCl (pH 7.6) 0.1 mM dithiothreitol buffer, 0.5 mM NAD(P)H, 55 mM acetaldehyde, and 2 – 50 μ l cell extract in 1.2 ml total volume. Decrease in absorbance was monitored at 340 nm to follow NAD(P)H oxidation (extinction coefficient 6.22 mM⁻¹ cm⁻¹) using an Agilent 8453 UV-Vis spectrophotometer with Peltier controlled heating set at 55°C. Protein concentration was determined using the Bradford method.

Homology modelling of *C. thermocellum*

AdhE. Using the protein sequence of the alcohol dehydrogenase (AdhE) domain of *C. thermocellum* ALDH/ADH, homology models of wild-type and double-mutant AdhE were constructed. The HHPRED webserver (10, 11), part of the Bioinformatics Toolkit webserver (12), was used to perform multiple sequence alignments of the *C. thermocellum* AdhE sequence to potential structural templates available in the Protein Data Bank (13). The 2.7 Å resolution X-ray structure of 1,3-propanediol dehydrogenase from *Klebsiella pneumoniae* (PDB ID 3BFJ) (14) and the 1.3 Å X-ray structure of Fe-containing alcohol dehydrogenase from *Thermotoga maritima* Tm0920 (PDB ID 1O2D)(15) were selected as templates based on their homology to AdhE, and inclusion of NAD(P), Fe, or both in the structures. The program MODELLER (16-19) was used to construct homology structures including the NAD cofactor and Fe ligand, and resulting models were assessed using the Discrete Optimized Protein Energy (DOPE) method (20). The fully automated I-TASSER webserver (21) was also used to construct a homology model, and it was found that MODELLER produced structures with a more favorable (i.e. lower) DOPE score if the I-TASSER model was included as an additional template structure. The reason this step improved the model is because the AdhE sequence has two regions that are not covered by either of the two PDB templates, and I-TASSER includes secondary structure prediction for sequences with no structural template. These regions are positioned on the same face of the protein as the N-terminus, which

would be joined to the C-terminus of the ALDH domain in the full-length ALDH/ADH. Thus, it is likely that these two regions interact with ALDH, providing a possible explanation for why they are not present in other single-domain alcohol dehydrogenases. To provide an additional level of validation of the homology structures, the MolProbity server (22, 23) was used to perform a Ramachandran analysis on the final models. For wild-type AdhE, 92% of all residues were found to be in favored regions, and 98.3% were in allowed regions. Seven residues were found to be outliers, an acceptable result considering the somewhat low sequence identity between the AdhE sequence and structural templates.

Molecular dynamics simulations

Molecular dynamics simulations were performed on the homology models on wild-type and double mutant (P704L, H734R) AdhE using the program GROMACS (24) with the CHARMM 27 force field (25) and TIP3P water model (26). Previously Lennard-Jones parameters for Fe(II) were used (27). Energy minimization was performed on the homology structures using the steepest descent method for 1000 steps, and then each protein was solvated in a rectangular water box of with a minimum of 10 Å from the surface of the protein to the edge of the solvent box. Sodium cations were added to neutralize the charge of the system. Periodic boundary conditions were imposed, and the Particle Mesh Ewald (PME) method (28, 29) was used to describe long-range electrostatic interactions. MD simulations were carried out with an integration time step of 2 fs. To reach the target temperature (298 K) and pressure (1 bar), the Berendsen method was used with a relaxation time of 0.1 ps (30). After a 1 ns equilibration, production simulations were performed in the NPT ensemble using the Nose-Hoover thermostat (31, 32) and the Parrinello-Rahman barostat (33, 34) with relaxation times of 1.0 ps. The production run was carried out for 10 ns, and coordinates were saved every 1 ps for analysis.

References

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Table S1. High confidence differences identified in wild-type strain by pyrosequencing

Replicon	Start Pos	End Pos	Reference sequence	Observed sequence	Region	Change	Codon number	Orig_AA	New_AA	Locus_Tag	Gene start	Gene stop	Strand	Product	Pyrosequencing results						
															# reads fwd	# reads rev	#Var	#Tot	(%)	Found in EA	
CP000568	3073025	3073025	C	T	INTERGENIC	Mul										10	12	22	22	100	
CP000568	1463053	1463053	T	C	NON	One	391 T	A		Cthe1221	1455455	1464223 -		glycosyltransferase 36		9	17	26	26	100	1463053
CP000568	3833351	3833351	T	C	NON	Mul	274 W	R		Cthe3232	3832532	3837541 +		YD repeat protein		21	13	34	34	100	3833351
CP000568	2315283	2315283	C	-	NON	Del	334 G	-		Cthe1939	2314949	2316283 -		magnesium transporter		18	16	34	35	97	
CP000568	2088722	2088722	G	-	NON	One	149 A	V		Cthe1766	2088334	2089167 -		glutamate 5-kinase		15	20	35	40	87	
CP000568	3373938	3373938	A	G	SYN	One	226 G	G		Cthe2855	3373261	3374499 +		conserved hypothetical protein		12	14	26	51	51	3373938
CP000568	3373944	3373944	T	G	SYN	One	228 D	D		Cthe2855	3373261	3374499 +		conserved hypothetical protein		13	14	27	53	51	
CP000568	1198646	1198646	G	-	NON	One	116 S	L		Cthe1000	1198156	1198992 -		phosphatidate cytidylyltransferase		8	2	10	21	48	
CP000568	3342625	3342627	TGA	-	NON	Del	560 N	-		Cthe2825	3340946	3342793 +		conserved hypothetical protein precursor		11	9	20	47	43	3342625
CP000568	3342638	3342638	G	A	NON	Mul	565 E	K		Cthe2825	3340946	3342793 +		conserved hypothetical protein precursor		11	10	21	49	43	3342638
CP000568	2397516	2397516	A	C	NON	Mul	60 M	R		Cthe2018	2396459	2397694 -		conserved hypothetical protein		17	10	27	65	42	2397516
CP000568	2397410	2397410	T	C	SYN	One	95 E	E		Cthe2018	2396459	2397694 -		conserved hypothetical protein		12	11	23	56	41	2397410
CP000568	2397503	2397509	ATTCATG	GTCCATA	NON	One	64 N	-		Cthe2018	2396459	2397694 -		conserved hypothetical protein		16	10	26	64	41	2397503
CP000568	2371533	2371535	GCC	-	NON	Del	113 A	-		Cthe1900	2371523	2371870 -		hypothetical protein		1	3	4	10	40	2371533
CP000568	1198190	1198190	G	A	NON	One	268 P	L		Cthe1000	1198156	1198992 -		phosphatidate cytidylyltransferase		6	8	14	35	40	
CP000568	1776760	1776760	G	A	NON	One	189 P	S		Cthe1457	1776662	1777324 -		polar amino acid ABC transporter, inner membrane		7	7	14	35	40	
CP000568	2397524	2397526	TTG	GTC	NON	One	57 Q	-		Cthe2018	2396459	2397694 -		conserved hypothetical protein		17	10	27	67	40	2397524
CP000568	488360	488360	-	GATATGCAGGAATATACTAC	NON	Ins	191 K	-		Cthe0392	487789	488833 +		inner-membrane translocator		7	9	16	41	39	
CP000568	3342604	3342621	GATAGGTGATAAAACAGG	AAAGGGTGTAGATCAGGGGA	NON	One	553 L	-		Cthe2825	3340946	3342793 +		conserved hypothetical protein precursor		7	8	15	39	38	3342604
CP000568	2397551	2397554	TTGG	CTGA	NON	Mul	48 Q	-		Cthe2018	2396459	2397694 -		conserved hypothetical protein		16	8	24	64	37	2397551
CP000568	956162	956162	G	AGT	NON	One	234 S	-		Cthe0792	956159	956863 -		Protein of unknown function UPF0001		8	1	9	26	35	
CP000568	2219915	2219915	-	A	INTERGENIC	Ins										2	8	10	30	33	
CP000568	2397590	2397590	G	A	SYN	Mul	35 D	D		Cthe2018	2396459	2397694 -		conserved hypothetical protein		10	4	14	43	33	
CP000568	3342592	3342592	G	A	SYN	One	549 S	S		Cthe2825	3340946	3342793 +		conserved hypothetical protein precursor		7	7	14	42	33	3342592
CP000568	582135	582135	A	G	SYN	One	66 K	K		Cthe0467	581938	582729 +		Flagellar biosynthesis/type III secretory		2	6	8	25	32	
CP000568	2394647	2394647	C	T	INTERGENIC	Mul										2	7	9	28	32	
CP000568	2796652	2796652	-	A	NON	Ins	730 L	F		Cthe2346	2795812	2798841 -		O-antigen polymerase		7	3	10	32	31	
CP000568	2397467	2397467	A	G	SYN	One	76 P	P		Cthe2018	2396459	2397694 -		conserved hypothetical protein		12	8	20	64	31	
CP000568	3373687	3373689	TCC	GCT	NON	One	143 S	-		Cthe2855	3373261	3374499 +		conserved hypothetical protein		5	4	9	30	30	
CP000568	2397277	2397277	A	G	SYN	Mul	140 L	L		Cthe2018	2396459	2397694 -		conserved hypothetical protein		3	5	8	28	29	2397277
CP000568	3373707	3373707	T	C	SYN	Mul	149 N	N		Cthe2855	3373261	3374499 +		conserved hypothetical protein		5	3	8	28	29	3373707
CP000568	1563377	1563377	-	T	NON	Ins	379 A	T		Cthe1286	1562982	1564511 -		peptidase S1 and S6, chymotrypsin/Hap		7	2	9	31	29	
CP000568	3342959	3342959	A	G	SYN	One	48 Q	Q		Cthe2826	3342816	3344054 +		conserved hypothetical protein		7	4	11	41	27	3342959
CP000568	1276285	1276285	-	A	NON	Ins	88 D	Y		Cthe1069	1276058	1276546 -		protein of unknown function UPF0054		1	2	3	12	25	
CP000568	2290234	2290234	G	CGT	NON	One	13 G	-		Cthe1919	2289574	2290272 -		MgTC/SapB transporter		1	3	4	16	25	
CP000568	3374106	3374106	A	G	SYN	One	282 R	R		Cthe2855	3373261	3374499 +		conserved hypothetical protein		2	7	9	36	25	3374106
CP000568	311030	311030	C	T	SYN	One	99 G	G		Cthe0253	310734	311723 +		ATPase associated with various cellular		3	3	6	25	24	
CP000568	2110164	2110164	G	CGA	NON	One	42 S	-		Cthe1788	2110040	2111296 +		glycosyl transferase, family 2		1	5	6	25	24	
CP000568	3327479	3327482	TTAA	AAAT	INTERGENIC	One										4	2	6	25	24	3327479
CP000568	488356	488356	T	ATA	NON	One	190 S	-		Cthe0392	487789	488833 +		inner-membrane translocator		5	4	9	39	23	
CP000568	3342563	3342563	A	G	NON	One	540 T	A		Cthe2825	3340946	3342793 +		conserved hypothetical protein precursor		5	3	8	36	22	3342563
CP000568	829398	829398	-	TA	INTERGENIC	Ins										2	2	4	19	21	
CP000568	1597777	1597777	G	A	NON	One	150 P	S		Cthe1313	1597028	1598224 -		phosphopantothenoylcysteine		5	1	6	28	21	
CP000568	2565681	2565682	AT	TA	INTERGENIC	One										1	5	6	29	21	
CP000568	2197451	2197451	C	T	INTERGENIC	One										5	3	8	38	21	
CP000568	3342559	3342559	T	G	SYN	One	538 L	L		Cthe2825	3340946	3342793 +		conserved hypothetical protein precursor		5	3	8	38	21	3342559
CP000568	3342553	3342553	G	A	SYN	One	536 A	A		Cthe2825	3340946	3342793 +		conserved hypothetical protein precursor		4	3	7	36	19	3342553
CP000568	3374120	3374124	GATTC	AGTTT	NON	One	287 R	-		Cthe2855	3373261	3374499 +		conserved hypothetical protein		2	5	7	36	19	
CP000568	3112041	3112041	G	A	SYN	Mul	467 R	R		Cthe2632	3110641	3114177 +		transcription-repair coupling factor		1	3	4	22	18	
CP000568	2721434	2721435	TC	GTT	INTERGENIC	One										1	5	6	33	18	
CP000568	975105	975106	TG	CT	NON	One	145 E	-		Cthe0806	972830	975538 -		PAS/PAC sensor hybrid histidine kinase		3	1	4	23	17	
CP000568	3281497	3281498	GG	AA	INTERGENIC	One										2	2	4	23	17	
CP000568	1221337	1221337	G	A	NON	One	102 T	I		Cthe1020	1220262	1221641 -		extracellular solute-binding protein, family 1		1	4	5	29	17	
CP000568	1155791	1155791	C	ACT	INTERGENIC	One										2	1	3	19	16	
CP000568	835435	835435	T	C	NON	One	48 V	A		Cthe0679	835293	836507 +		Serine-type D-Ala-D-Ala carboxypeptidase		2	2	4	25	16	
CP000568	2451889	2451889	G	A	SYN	One	94 S	S		Cthe2060	2451337	2452170 -		RNA methyltransferase, TrmH family, group 3		3	3	6	37	16	
CP000568	2019375	2019375	G	A	NON	One	139 S	F		Cthe1695	2018315	2019790 -		Radical SAM		1	3	4	26	15	
CP000568	149324	149324	G	CGA	NON	One	77 S	-		Cthe0116	149095	150039 +		protein of unknown function DUF199		1	2	3	21	14	
CP000568	2199693	2199693	G	A	NON	One	60 A	V		Cthe1853	2199671	2199871 -		cold-shock DNA-binding domain protein		1	2	3	23	13	
CP000568	3373046	3373052	TTAGTGG	CTAAAGGGT	NON	One	553 L	-		Cthe2854	3371390	3373237 +		conserved hypothetical protein precursor		2	2	4	30	13	3373046
CP000568	3373064	3373067	AGAA	GGGG	NON	Mul	559 R	-		Cthe2854	3371390	3373237 +		conserved hypothetical protein precursor		2	2	4	31	13	
CP000568	1477522	1477522	G	T	NON	One	179 K	N		Cthe1233	1476986	1477525 +		hypothetical protein		2	1	3	24	12	

Table S2. High confidence differences identified in strain EA by pyrosequencing

Replicon	Start Pos	End Pos	Reference sequence	Observed sequence	Region	Change	Codon number	Orig. AA	New AA	Locus Tag	Gene start	Gene stop	Strand	Product	Pyrosequencing results				Found in WT
															# reads fwd	# reads rev	#Var	#Tot	
CP000568	2239488	2239488 T		C	SYN	One	205 E	G	Che1886	2232455	2240081-		Integrase, catalytic region	2	1	3	3	100	
CP000568	3373201	3373201 AAGT		G	NON	Mul	537	Y	Che2854A	3373227	3373227	1	conserved hypothetical protein precursor	1	3	4	4	100	
CP000568	3373707	3373707 T		C	NON	Mul	149 N	N	Che2855	3373261	3374490+	1	conserved hypothetical protein	1	3	4	4	100	3373707
CP000568	3379264	3379264 G		A	NON	One	359 D	N	Che2860	3378190	3379900+	1	hypothetical protein precursor	1	3	4	4	100	
CP000568	3786797	3786797 T		GCA	INTERGENIC	One						3		1	4	4	4	100	
CP000568	3800424	3800424 A		G	NON	One	277 V	A	Che3208	3800183	3801253-	2	Integrase, catalytic region	2	2	4	4	100	
CP000568	3625227	3625227 A		A	SYN	One	1433 P	P	Che3078	3625229	3632170+	4	cellulosome anchoring protein, cohesin region	4	1	5	5	100	
CP000568	3786785	3786793 GAAGCTATG		T	INTERGENIC	One						2		2	5	5	100		
CP000568	157500	157500 A		G	NON	One	350 R	R	Che0128	156451	157563+	4	Integrase, catalytic region	4	2	6	6	100	
CP000568	3373046	3373067 TTATGGGGTTCGCTCAAGA		G	SYN	One	553 L	-	Che2854	3371390	3373237+	4	conserved hypothetical protein precursor	4	3	7	7	100	3373046
CP000568	3379279	3379279 A		G	NON	One	364 T	A	Che2860	3378190	3379900+	3	hypothetical protein precursor	3	4	7	7	100	
CP000568	816559	816559 G		A	SYN	One	252 P	P	Che0661	815804	817519+	1	Ricin B lectin	1	3	8	8	100	
CP000568	2245692	2245692 A		G	NON	Mul	400 C	C	Che1890	2244759	2246891+	4	cellulosome enzyme, dockerin type I	4	4	8	8	100	
CP000568	2245718	2245720 TTG		CTT	NON	Mul	392 N	-	Che1890	2244759	2246891+	4	cellulosome enzyme, dockerin type I	4	4	8	8	100	
CP000568	3351245	3351245 A		G	SYN	One	358 E	E	Che2834	3350172	3351857+	4	conserved hypothetical protein precursor	4	4	8	8	100	
CP000568	3790320	3790395 AACTAGATTGCGAAAAGCCTGCCACATTCGCAGTGCACAAGGTGTTGAAGTGGACTTCCAGTAAACAAAGGATTTG		T	INTERGENIC	Del						4		4	8	8	100		
CP000568	1675440	1675440 C		C	SYN	One	511 T	T	Che1373	1671219	1676972-	4	YD repeat protein	4	4	8	8	100	
CP000568	1675446	1675446 C		C	SYN	One	509 A	A	Che1373	1671219	1676972-	4	YD repeat protein	4	5	9	9	100	
CP000568	1855353	1855353 T		C	NON	One	214 Y	C	Che1529	1854740	1855992-	4	transposase	4	5	9	9	100	
CP000568	3379321	3379321 A		G	NON	One	378 T	A	Che2860	3378190	3379900+	4	hypothetical protein precursor	4	5	9	9	100	
CP000568	3379335	3379335 C		G	NON	One	382 C	C	Che2860	3378190	3379900+	4	hypothetical protein precursor	4	5	9	9	100	
CP000568	3623485	3623487 TCC		GAA	NON	One	1324 S	-	Che3077	3619516	3625077+	5	cellulosome anchoring protein, cohesin region	5	4	9	9	100	
CP000568	5374106	5374106 A		G	SYN	One	282 R	R	Che2855	3373261	3374490+	5	conserved hypothetical protein	5	5	10	10	100	3374106
CP000568	628597	628597 G		A	NON	One	326 A	T	Che0514	627622	628737+	5	DNA methylase N-4-N-6	5	5	10	10	100	
CP000568	1231266	1231266 G		A	NON	One	272 A	V	Che1029	1231004	1232080-	4	phosphate acetyltransferase	4	6	10	10	100	
CP000568	2137485	2137485 G		A	SYN	Mul	663 T	T	Che1806	2132940	2139473-	3	cellulosome enzyme, dockerin type I	3	7	10	10	100	
CP000568	2137527	2137527 T		C	SYN	One	649 L	L	Che1806	2132940	2139473-	3	cellulosome enzyme, dockerin type I	3	5	10	10	100	
CP000568	3379345	3379345 A		C	NON	One	336 R	R	Che2860	3378190	3379900+	4	hypothetical protein precursor	4	6	10	10	100	
CP000568	56259	56259 A		G	NON	One	629 P	P	Che0043	54373	56601+	3	glycoside hydrolase, family 9	3	8	11	11	100	
CP000568	533551	533551 A		G	NON	One	734 H	R	Che0423	531351	533972+	6	iron-containing alcohol dehydrogenase	6	5	11	11	100	
CP000568	1460496	1460496 T		C	NON	One	1243 E	G	Che1221	1455455	1464223-	4	glycosyltransferase 36	4	5	11	11	100	
CP000568	1897171	1897171 G		A	NON	One	211 R	Q	Che1671	1996540	1998108+	7	Recombinase	7	7	14	14	100	
CP000568	2012225	2012225 T		C	INTERGENIC	One						7		7	4	11	11	100	
CP000568	2137509	2137509 C		T	SYN	One	655 S	S	Che1806	2132940	2139473-	5	cellulosome enzyme, dockerin type I	5	6	11	11	100	
CP000568	3526732	3526732 C		G	NON	One	77 C	F	Che3003	3525027	3526961-	6	hydrogenases, Fe-only	6	5	11	11	100	
CP000568	3532242	3532242 A		G	NON	One	116 F	S	Che3008	3531917	3532588-	7	Superoxide dismutase	7	4	11	11	100	
CP000568	401007	401007 C		T	NON	One	124 G	S	Che0317	400705	401376+	4	hypothetical protein	4	8	12	12	100	
CP000568	410756	410756 A		G	SYN	One	53 T	T	Che2855	410598	412538+	5	NAD+ synthetase	5	7	12	12	100	
CP000568	537435	537435 A		G	SYN	One	248 K	K	Che0427	536692	537861+	4	serine phosphatase	4	8	12	12	100	
CP000568	763262	763262 G		A	NON	One	61 P	L	Che0622	762637	763443+	5	methylthioadenosine phosphorylase	5	7	12	12	100	
CP000568	939962	939962 A		-	NON	Del	660 F	-	Che0777	939329	941941-	3	DNA mismatch repair protein MutS	3	9	12	12	100	
CP000568	950504	950504 G		A	SYN	One	74 V	V	Che0789	954484	955275-	8	DNA-binding S4	8	4	12	12	100	
CP000568	1311107	1311107 A		G	NON	One	324 H	K	Che1101	1310786	1312078+	8	hypothetical protein	8	7	12	12	100	
CP000568	1606832	1606832 T		C	NON	One	24 E	G	Che1323	1606222	1606902-	6	GpC protein	6	7	12	12	100	
CP000568	1818170	1818170 C		T	SYN	One	461 A	A	Che1498	1817744	1819552-	9	methyl-accepting chemotaxis sensory transducer	9	3	12	12	100	
CP000568	2504381	2504381 T		C	NON	Mul	807 H	R	Che2109	2504263	2506800-	4	copper amine oxidase-like	4	8	12	12	100	
CP000568	37536	37536 G		T	NON	One	223 A	S	Che0030	36870	37580+	6	phosphatidate cytidyltransferase	6	7	13	13	100	
CP000568	462588	462588 G		G	SYN	One	243 V	V	Che0367	461825	462694+	5	NAD(P)-dependent coenzyme F420-dependent	5	9	13	13	100	
CP000568	535432	535432 C		T	NON	One	135 L	L	Che0426	535029	536690+	9	putative PAS/PAC sensor protein	9	4	13	13	100	
CP000568	921216	921216 T		C	NON	One	174 N	D	Che0755	920548	921735-	9	aminotransferase, class I and II	9	4	13	13	100	
CP000568	1194244	1194244 G		A	SYN	One	350 L	L	Che0997	1194233	1195291-	5	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate	5	8	13	13	100	
CP000568	1633288	1633288 G		A	NON	One	281 P	L	Che1341	1632612	1634129-	4	Radical SAM	4	9	13	13	100	
CP000568	1713360	1713360 TTGATAAAATAAACAAATTTCTGTGTAGATAGTTTTATCGTACCTATGAGGAAATGAAACT		T	INTERGENIC	Del						8		8	5	13	13	100	
CP000568	1770389	1770389 T		C	NON	One	254 E	G	Che1450	1770387	1771149-	8	conserved hypothetical protein	8	5	13	13	100	
CP000568	1872581	1872581 C		T	INTERGENIC	One						8		8	9	13	13	100	
CP000568	1953853	1953853-		G	SYN	Ins	44 G	G	Che1625	1953574	1953984-	7	phage protein, HK97 gp10 family	7	6	13	13	100	
CP000568	2491691	2491691 C		T	SYN	One	43 S	S	Che2095	2491052	2491819-	8	hydrolase, TaD family	8	5	13	13	100	
CP000568	2595580	2595580 T		C	NON	One	186 D	G	Che2179	2593348	2596116-	5	Pectate lyase/Amb allergen	5	8	13	13	100	
CP000568	164619	164619 A		G	INTERGENIC	One						10		4	14	14	100		
CP000568	356311	356311 C		T	SYN	One	389 V	V	Che0287	355145	358627+	9	multi-sensor hybrid histidine kinase	9	5	14	14	100	
CP000568	432634	432634 A		G	SYN	One	137 A	A	Che0343	432224	432826+	7	flavin reductase-like, FMN-binding	7	6	14	14	100	
CP000568	488363	488391 TATCGAGGAATATATACTACGTGGGAAGC		-	NON	Del	1921	-	Che0392	487789	488838+	8	inner-membrane translocator	8	7	14	14	100	
CP000568	903250	903250 V		G	SYN	One	270 V	V	Che0486	903047	903496+	5	GTP-binding signal recognition particle SRP54	5	9	14	14	100	
CP000568	933177	933177 A		G	NON	One	208 T	T	Che0770	932459	933790-	3	signal recognition particle protein	3	11	14	14	100	
CP000568	1012867	1012867 G		A	NON	One	146 V	V	Che0831	1012416	1013303-	6	Polyprenyl synthetase	6	8	14	14	100	
CP000568	1088																		

CP000568	1678574	1678574 C	T	NON	One	322 G	S	Che1375	1678188	1679537	aspartate kinase	8	9	17	17	100	
CP000568	1778192	1778192 A	-	NON	Del	9 L	-	Che1458	1777408	1778217	extracellular solute-binding protein, family 3	11	6	17	17	100	
CP000568	1821198	1821198 T	-	NON	One	216 W	R	Che1499	1820553	1822307+	ammonium transporter	7	7	17	17	100	
CP000568	2141861	2141861 G	A	NON	One	307 A	V	Che1807	2141116	2142780-	conserved hypothetical protein	9	8	17	17	100	
CP000568	2460536	2460536 T	-	INTERGENIC	One							7	10	17	17	100	
CP000568	2657596	2657596 C	T	NON	One	84 G	D	Che2229	2656764	2657616	N-acetylneuraminate synthase	7	10	17	17	100	
CP000568	2661872	2661872 A	G	SYN	One	103 L	L	Che2234	2661795	2662178-	hypothetical protein	10	7	17	17	100	
CP000568	2775727	2775727 T	C	NON	One	153 H	R	Che2329	2775348	2776184-	hypothetical protein	7	10	17	17	100	
CP000568	2920132	2920132 T	C	NON	One	193 P	P	Che2448	2919555	2920538+	inner-membrane translocator	7	10	17	17	100	
CP000568	3458321	3458321 G	A	NON	One	209 A	T	Che2943	3457697	3458914+	ABC-2 type transporter	8	9	17	17	100	
CP000568	3496333	3496333 G	A	NON	One	245 E	K	Che2977	3495704	3496701+	MAE3L putative	7	17	17	100		
CP000568	3752468	3752468 A	G	NON	One	181 T	T	Che3174	3752151	3752520-	conserved hypothetical protein	6	11	17	17	100	
CP000568	3373938	3373938 A	G	SYN	One	226 G	G	Che2855	3373261	3374499+	conserved hypothetical protein	6	12	18	18	100	
CP000568	1463053	1463053 T	C	NON	One	391 T	A	Che1221	1455455	1464223-	glycosyltransferase 36	7	11	18	18	100	
CP000568	232355	232355 A	G	NON	One	523 T	A	Che0191	230789	232588+	proteinase inhibitor I4, serpin	10	8	18	18	100	
CP000568	580832	580832 C	G	A	NON	One	4 G	S	Che0466	580923	581945+	flagellar motor switch protein FIG	7	11	18	18	100
CP000568	881843	881843 A	A	INTERGENIC	One							7	11	18	18	100	
CP000568	815352	815352 C	A	T	NON	One	692 T	M	Che0660	813278	815599+	glycoside hydrolase, family 81	11	7	18	18	100
CP000568	915475	915475 A	G	NON	One	213 V	A	Che0749	915285	916112-	binding-protein-dependent transport systems	12	6	18	18	100	
CP000568	1072740	1072740 C	T	NON	One	466 E	K	Che0896	1072333	1074135-	DNA primase	6	12	18	18	100	
CP000568	1400010	1400010 G	A	NON	One	177 I	I	Che1172	1399482	1400630+	protein of unknown function DUF362	9	9	18	18	100	
CP000568	1552089	1552089 C	T	NON	One	152 H	K	Che1275	1521754	1552191-	H+-ATPase subunit H	8	10	18	18	100	
CP000568	1625686	1625686 C	G	NON	One	409 I	I	Che1334	1624461	1626023+	FHA domain containing protein	10	8	18	18	100	
CP000568	1645595	1645595 T	C	SYN	One	21 V	V	Che1352	1644326	1645657-	UDP-glucose 6-dehydrogenase	10	8	18	18	100	
CP000568	1734027	1734027 G	C	NON	One	33 Q	R	Che1413	1732208	1734124-	hypothetical protein	12	6	18	18	100	
CP000568	1943396	1943396 T	C	NON	One	461 D	G	Che1613	1942044	1944767-	glycosyl hydrolase-like	10	8	18	18	100	
CP000568	2033223	2033223 G	A	NON	One	412 T	T	Che1609	2031877	2034156-	Phage-related protein-like	9	9	18	18	100	
CP000568	2139279	2139279 G	A	NON	One	65 G	G	Che1806	2132940	2139473-	cellulose enzyme, dockerin type I	10	8	18	18	100	
CP000568	2419590	2419590 C	T	INTERGENIC	One							10	8	18	18	100	
CP000568	2739844	2739912 TTTCAATTCCTACAGTACGATAAAAACCCATTAGATAGAGAATTGAAAAATCGCTGCAAATGATA	-	INTERGENIC	Del	317 A	T	Che2550	3019150	3020133-	glycosyltransferase sugar-binding region	8	10	18	18	100	
CP000568	3019185	3019185 C	T	NON	Mul	22 R	R	Che2574	3046452	3047264+	binding-protein-dependent transport systems	15	3	18	18	100	
CP000568	3046517	3046517 T	C	SYN	Ins	62 G	G	Che2808	3309546	3310583+	transcriptional regulator, LacI family	9	9	18	18	100	
CP000568	3309731	3309731 C	A	NON	One	17 A	V	Che2917	3440034	3440432+	ribosomal protein S8	10	8	18	18	100	
CP000568	3440083	3440083 C	T	NON	One	271 T	A	Che3096	3653111	3654046-	hypothetical protein	3	15	18	18	100	
CP000568	3653236	3653236 T	-	INTERGENIC	Del							10	9	19	19	100	
CP000568	92472	92472 A	-	STOP	One	328 Q	*	Che0208	252608	254899+	single-stranded-DNA-specific exonuclease RecJ	7	12	19	19	100	
CP000568	253589	253589 C	G	SYN	One	66 S	S	Che0298	372821	374623+	methyl-accepting chemotaxis sensory transducer	9	10	19	19	100	
CP000568	373018	373018 A	G	NON	One	144 A	V	Che0406	507482	508219-	conserved hypothetical protein	9	10	19	19	100	
CP000568	507789	507789 G	A	NON	Mul	119 N	D	Che0511	623795	625117+	histidine kinase	11	8	19	19	100	
CP000568	624149	624149 A	G	NON	One	303 M	V	Che0561	689831	689977+	AbpE-like lipoprotein	10	9	19	19	100	
CP000568	689837	689837 A	-	INTERGENIC	Del							13	6	19	19	100	
CP000568	1031281	1031281 A	G	SYN	One	179 A	A	Che1077	1284358	1284987+	hypothetical protein	7	10	19	19	100	
CP000568	1284894	1284894 C	T	NON	One	75 C	C	Che1776	2098068	2098553-	protein of unknown function UPF0079	10	9	19	19	100	
CP000568	2098330	2098330 T	C	NON	Ins	62 I	I	Che1811	2147403	2147777-	transcriptional repressor, CopY family	9	10	19	19	100	
CP000568	2147594	2147594- A	A	NON	One	125 D	N	Che1889	2238441	2239103+	phage integrase-like SAM-like	9	10	19	19	100	
CP000568	2238813	2238813 G	A	SYN	One	563 T	T	Che1917	2285111	2287708-	ATPase P-type (transporting), HAD superfamily,	12	7	19	19	100	
CP000568	2478733	2478733 C	T	NON	One	745 N	D	Che2086	2478446	2480955-	peptidase U32	12	7	19	19	100	
CP000568	2713030	2713030 G	A	NON	One	146 R	W	Che2282	2712653	2713465-	MCP methyltransferase, CheR-type	8	11	19	19	100	
CP000568	3001848	3001848 G	A	NON	One	103 D	N	Che2532	3001542	3002390+	sulfate ABC transporter, inner membrane subunit	11	8	19	19	100	
CP000568	3094111	3094111 G	A	NON	One	1103 D	N	Che2612	3087105	3090941+	Fibronectin, type III	9	10	19	19	100	
CP000568	3132489	3132489 C	T	NON	One	368 P	P	Che2650	3131748	3133571-	polysaccharide biosynthesis protein CapD	8	9	19	19	100	
CP000568	3227821	3227821 G	A	NON	One	258 V	I	Che2736	3227050	3228756+	phosphoenolpyruvate-protein phosphotransferase	11	8	19	19	100	
CP000568	3236755	3236755 A	G	INTERGENIC	One							11	8	19	19	100	
CP000568	3811733	3811733 A	G	NON	One	37 D	G	Che3217	3811624	3812916+	hypothetical protein	7	12	19	19	100	
CP000568	136133	136133 C	G	NON	One	35 D	G	Che0104	136300	137127+	riboflavin biosynthesis protein RibD	8	12	20	20	100	
CP000568	351245	351245 G	A	SYN	One	143 P	P	Che0284	350817	352175+	protein of unknown function DUF815	11	7	20	20	100	
CP000568	556152	556152 C	T	INTERGENIC	One							9	11	20	20	100	
CP000568	613076	613076 A	G	SYN	One	44 S	S	Che0502	612945	613811+	hypothetical protein	11	9	20	20	100	
CP000568	812836	812836 A	-	NON	Del	1 L	-	Che0659	812448	812837-	conserved hypothetical protein	11	9	20	20	100	
CP000568	1332697	1332697 T	C	SYN	One	127 S	S	Che1116	1330912	1333077-	HMG-I and HMG-V, DNA-binding	10	10	20	20	100	
CP000568	1530897	1530897 T	C	NON	One	1431 M	M	Che1302	1579458	1581125-	conserved hypothetical protein	9	11	20	20	100	
CP000568	1583731	1583731 T	C	NON	One	280 K	E	Che1304	1583135	1584508-	Pf6H-like protein	11	9	20	20	100	
CP000568	1796657	1796657 T	C	INTERGENIC	One							6	14	20	20	100	
CP000568	2168008	2168008 T	C	NON	One	485 D	G	Che1829	2166165	2169461-	Chromosome segregation ATPases-like	11	11	20	20	100	
CP000568	2386244	2386244 A	G	NON	Mul	136 L	F	Che2006	2386594	2386649-	hypothetical protein precursor	5	15	20	20	100	
CP000568	2517922	2517922 T	C	NON	One	2751 V	V	Che2121	2516821	2518814+	glycoside hydrolase, family 10	11	9	20	20	100	
CP000568	2892640	2892640 C	T	NON	One	58 P	S	Che2263	2892469	2892936+	H+-transporting two-sector ATPase, C subunit	6	14	20	20	100	
CP000568	2971415	2971415 T	C	NON	One	25 D	G	Che2504	2970199	2971488-	3D	9	11	20	20	100	
CP000568	3031718	3031718 G	A	STOP	One	156 W	*	Che2560	3031251	3031799+	dTDP-4-dehydrohamose 3,5-epimerase	11	9	20	20	100	
CP000568	3064045	3064045 G	A	INTERGENIC	One							11	9	20	20	100	
CP000568	3082151	3082151 A	G	SYN	One	9 V	V	Che2803	3304204	3304983+	binding-protein-dependent transport systems	5	15	20	20	100	
CP000568	3304230	3304230 A	G	NON	One	51 D	N	Che2927	3445723	3445941+	translation initiation factor IF-1	11	9	20	20	100	
CP000568	345873	345873 G	A	NON	One	224 F	V	Che2966	3484611	3485357+	hypothetical protein	10	9	20	20	100	
CP000568	3485280	3485280 T	G	NON	One	494*	Q	Che3004	3526995	3528476-	ferredoxin	13	7	20	20	100	
CP000568	3626997	3626997 A	G	NON	One	167 D	D	Che3096	3607660	3608385+	ABC transporter related	9	11	20	20	100	
CP000568	3608159	3608159 A	G	SYN	One	178 V	V	Che3158	3678143	3677156+	conserved hypothetical protein	9	12	20	20	100	
CP000568	3731348	3731348 G	A	SYN	One	380 A	V	Che0071	3730815	3732743+	putative acrilonate hydratase	10	10	20	20	100	
CP000568	104530	104530 G	A	NON	One	102852	105668-	Che0071	102852	105668-	Cellulose 1,4-beta-cellobiosidase	10	11	21	21	100	
CP000568	531270	531270 T	C	NON	One	195 M	T	Che0422	530687	531370+	CoA-binding	13	8	21	21	100	
CP000568	632372	632372 C	G	NON	One	9 V	L	Che0517	632019	632387-	hypothetical protein	6	15	21	21	100	
CP000568	1080738	1080738 A	G	NON	One	34 L	F	Che0903	1073983	1080837-	protein-export membrane protein SecF	14	7	21	21	100	
CP000568	1113552	1113552 T	C	NON	One	959 T	A	Che0927	1112854	1116426-	chromosome segregation protein SMC	10	11	21	21	100	
CP000568	1141582	1141582 A	G	SYN	One	304 H	H	Che0952	114								

CP000568	385361	385361 A	G	SYN	One	75	G	Che0307	385137	385514+	conserved hypothetical protein	12	11	23	23	100
CP000568	556255	556255 A	G	INTERGENIC	One											100
CP000568	1015063	1015063 A	G	NON	One	76	S	Che0834	1014851	1015288-	NusB antitermination factor	7	16	23	23	100
CP000568	1771468	1771468 C	C	NON	One	120	R	Che1451	1771353	1771826-	GCN5-related N-acetyltransferase	14	9	23	23	100
CP000568	1917309	1917309 T	C	SYN	One	159	P	Che1586	1916130	1917785-	binding-protein-dependent transport systems	12	11	23	23	100
CP000568	2178645	2178645 T	C	SYN	One	23	S	Che1885	2175342	2178650-	Viral A-type inclusion protein repeat	10	13	23	23	100
CP000568	2210573	2210573 C	T	NON	One	240	N	Che1884	2209854	2210768+	acetylglutamate kinase	12	11	23	23	100
CP000568	2795536	2795536 G	A	SYN	One	73	T	Che2345	2794605	2795753+	DegT/DnrJ/EryC1/StrS aminotransferase	14	9	23	23	100
CP000568	2831640	2831640 T	T	NON	Del	951	-	Che2366	2831302	2831922-	single-stranded nucleic acid binding R3H	15	8	23	23	100
CP000568	2908769	2908769 C	T	NON	One	55	R	Che2436	2908696	2908932-	hypothetical protein	10	13	23	23	100
CP000568	3116739	3116739 C	T	NON	Del	801	L	Che2655	3116740	3116934+	pyridoxal 5'-phosphate oxidase-related, HcH4/H-pal aldolase	15	8	23	23	100
CP000568	3131416	3131416 C	G	SYN	One	39	Q	Che2649	3130705	3131532-	ATPase, P-type (transporting), HAD superfamily, hydrogenase expression/formation protein HyeP	10	13	23	23	100
CP000568	3147607	3147607 A	T	NON	One	97	A	Che3013	3537193	3538170-	Rubredoxin-type Fe(Cys)4 protein	10	13	23	23	100
CP000568	3537881	3537881 G	A	SYN	One	62	A	Che0063	96121	96456+	copper amine oxidase-like	17	7	24	24	100
CP000568	96306	96306 A	A	INTERGENIC	One	392	N	Che0744	909693	909809-		9	15	24	24	100
CP000568	908630	908630 G	A	NON	One	427	C	Che1041	1244775	1246175-	UDP-N-acetylmuramoylalanine-D-glutamate ligase	9	15	24	24	100
CP000568	1006265	1006265 T	A	SYN	One	334	V	Che1344	1635311	1637485-	(pp)Gpp synthetase I, Spo7RrelA	12	12	24	24	100
CP000568	1244897	1244897 G	T	STOP	One	363	W	Che1509	1833063	1834298-	protein of unknown function DUF438	8	16	24	24	100
CP000568	1636484	1636484 T	G	NON	One	146	S	Che1800	2126418	2127239+	Peptidoglycan-binding LysoM	11	13	24	24	100
CP000568	1833211	1833211 C	A	NON	One	151	L	Che1942	2317632	2318531+	hypothetical protein	12	14	24	24	100
CP000568	2126854	2126854 A	G	INTERGENIC	One											100
CP000568	2318380	2318380 G	A	SYN	One	390	D	Che2412	2879144	2881795+	SMC protein-like	12	12	24	24	100
CP000568	2362406	2362406 T	A	INTERGENIC	One											100
CP000568	2380313	2380313 C	T	NON	One	2737	D	Che0056	75384	89296+	Ig-like, group 2	14	10	24	24	100
CP000568	2398565	2398565 G	A	NON	One	59	A	Che0059	90979	92430+	type 3a cellulose-binding	15	10	25	25	100
CP000568	83602	83602 G	A	NON	Del	31	P	Che0175	211898	213034+	polysaccharide deacetylase	17	8	25	25	100
CP000568	89026	89026 G	A	SYN	One	151	S	Che0392	487789	488838+	inner-membrane translocator	14	11	25	25	100
CP000568	1157787	1157787 T	T	NON	One	682	E	Che0968	1157710	1159830-	UvrD/REP helicase	13	12	25	25	100
CP000568	1455798	1455798 C	T	NON	One	2809	A	Che1221	1455455	1464223+	glycosyltransferase 36	11	14	25	25	100
CP000568	1612520	1612520 T	C	SYN	One	244	P	Che1328	1611804	1613081-	Stage II sporulation P	14	11	25	25	100
CP000568	1894413	1894413 T	C	NON	One	49	S	Che1566	1894269	1895612+	Nitrogenase	12	13	25	25	100
CP000568	2087622	2087622 T	C	NON	One	215	L	Che1765	2087115	2088266-	hypothetical protein	11	14	25	25	100
CP000568	2421931	2421931 T	C	SYN	One	668	P	Che2038	2421511	2423934+	cellulosome enzyme, dockerin type I	10	15	25	25	100
CP000568	2449572	2449572 G	A	SYN	One	53	N	Che2058	2449203	2449730-	Appr-1-p processing	13	12	25	25	100
CP000568	2659299	2659299 T	A	NON	Del	27	Q	Che2054	2661795	2662178+	hypothetical protein	11	14	25	25	100
CP000568	2766062	2766062 G	-	NON	Del	195	N	Che2319	2765686	2766645+	restriction endonuclease (HaeIII)	16	9	25	25	100
CP000568	2837314	2837314 A	G	NON	One	34	R	Che2374	2837214	2838323+	DNA replication and repair protein RecF	13	12	25	25	100
CP000568	2995953	2995953 G	A	NON	One	236	S	Che2527	2995247	2996125+	porphobilinogen deaminase	9	16	25	25	100
CP000568	3304557	3304557 T	T	SYN	One	118	F	Che2803	3304204	3304983+	binding-protein-dependent transport systems	14	11	25	25	100
CP000568	3470913	3470913 A	C	NON	Del	65	Q	Che2951	3470720	3471445+	hypothetical protein	16	9	25	25	100
CP000568	1212630	1212630 T	T	INTERGENIC	One											100
CP000568	2160407	2160407 C	T	NON	One	768	D	Che1825	2159911	2162709-	multi-sensor hybrid histidine kinase	16	10	26	26	100
CP000568	2216332	2216332 C	T	NON	One	599	P	Che1868	2214537	2217740+	carbamoyl-phosphate synthase, large subunit	18	8	26	26	100
CP000568	2486234	2486234 G	A	INTERGENIC	One											100
CP000568	2843031	2843031 C	T	NON	One	165	P	Che2378	2842538	2843407+	parB-like partition proteins	14	12	26	26	100
CP000568	2884831	2884831 A	G	NON	One	201	D	Che2415	2884231	2884932+	cell wall hydrolase, SlaB	12	14	26	26	100
CP000568	2888660	2888660 C	T	NON	One	20	A	Che2418	2887674	2888717-	ATPase	15	11	26	26	100
CP000568	2900823	2900823 G	A	STOP	One	243	W	Che2427	2900096	2901052+	protein of unknown function DUF1385	15	11	26	26	100
CP000568	3029980	3029980 A	G	NON	One	49	D	Che2559	3029745	3031118+	DagT/DnrJ/EryC1/StrS aminotransferase	11	15	26	26	100
CP000568	3082999	3082999 T	G	SYN	One	289	L	Che2612	3082512	3090041+	Fibrinectin, type III	14	12	26	26	100
CP000568	3223060	3223060 C	T	NON	One	177	A	Che2730	3222531	3223733+	translation elongation factor Tu	13	13	26	26	100
CP000568	3400528	3400528 A	G	NON	One	502	R	Che2876	3399024	3401249+	ATP-dependent DNA helicase PcrA	12	14	26	26	100
CP000568	3420674	3420674 A	G	NON	One	911	V	Che2894	3420404	3420934+	conserved hypothetical protein	10	16	26	26	100
CP000568	3707682	3707682 A	G	INTERGENIC	One											100
CP000568	1390700	1390700 C	T	NON	Del	100	T	Che1165	1389543	1390754-	YbbR-like	16	10	26	26	100
CP000568	1715927	1715927 A	T	INTERGENIC	One	19	A									100
CP000568	1828037	1828037 T	C	NON	One	253	M	Che1504	1827280	1828092+	Linocin_M18 bacteriocin	12	15	27	27	100
CP000568	2903728	2903728 G	-	NON	Del	27	M	Che2430	2903648	2904967+	electron transport complex, RnfABCDGE type, C	14	13	27	27	100
CP000568	1506895	1506895-	T	SYN	Ins	26	K	Che1243	1506510	1506971-	GCN5-related N-acetyltransferase	15	13	28	28	100
CP000568	3086217	3086217 A	G	NON	Del	137	T	Che2611	3082209	3087095+	Fibrinectin, type III	16	12	28	28	100
CP000568	893075	893075 C	A	STOP	One	245	G	Che0735	893001	893907+	cellulosome anchoring protein, cohesin region	19	10	29	29	100
CP000568	15883	15883 T	A	NON	One	1110	A	Che0009	12555	18077+	YD repeat containing protein	17	13	30	30	100
CP000568	182829	182829 G	C	NON	One	175	A	Che0143	182307	183608+	Phosphorylase	19	12	31	31	100
CP000568	2432297	2432297 T	C	INTERGENIC	One											100
CP000568	3232523	3232523 G	A	SYN	One	110	Y	Che2815	3234051	3236652-	lysyl-tRNA synthetase	16	15	31	31	100
CP000568	2975548	2975548 A	A	NON	One	834	M	Che2506	2973047	2976098+	S-layer-like domain containing protein	13	19	32	32	100
CP000568	3031364	3031364 T	C	NON	One	38	A	Che2560	3031251	3031799+	dUDP-4-dehydrothiamine 3,5-epimerase	13	19	32	32	100
CP000568	2225182	2225182 C	T	NON	One	69	T	Che1873	2224043	2225386-	HMG-I and HMG-Y, DNA-binding	18	16	34	34	100
CP000568	2351019	2351019 C	T	SYN	One	83	E	Che1968	2348067	2351267-	cell division/ftsK/SpoIIIE	16	18	34	34	100
CP000568	3693510	3693510 C	T	INTERGENIC	One											100
CP000568	2947995	2947995 A	G	NON	One	361	S	Che2475	2946915	2948348+	phage portal protein, SPP1 family	18	26	44	44	100
CP000568	2943643	2943643 G	A	NON	One	333	G	Che2471	2942646	2943926+	conserved hypothetical protein	20	31	51	51	100
CP000568	2900141	2900141-	G	SYN	Ins	16	G	Che2427	2900096	2901052+	protein of unknown function DUF1385	16	15	31	31	97
CP000568	3240362	3240362 G	A	NON	One	42	Q	Che2746	3240238	3240708+	protein of unknown function DUF407	10	11	22	23	96
CP000568	3742942	3742942 C	T	SYN	One	37	V	Che3167	3742832	3743953+	glucose-1-phosphate adenylyltransferase, GlgD	11	12	22	23	96
CP000568	1561620	1561620 T	T	NON	Del	393	H	Che2085	1561401	1562786-	metal dependent phosphohydrolase	15	10	23	24	96
CP000568	2461889	2461889-	G	SYN	Ins	68	R	Che2069	2461687	2462463+	hypothetical protein	14	9	23	24	96
CP000568	536206	536206-	A	SYN	Ins	393	E	Che0426	536029	536699+	putative PAS/PAC sensor protein	14	10	24	25	96
CP000568	2116605	2116605 T	-	NON	Del	1231	-	Che1793	2116267	2116971+	protein of unknown function DUF1624	10	10	24	25	96
CP000568	2434214	2434214-	T	SYN	Ins	23	K	Che2044	2433860	2434282-	hypothetical protein	8	16	24	25	96
CP000568	187474	187474 A	G	NON	Del	187	K	Che0047	187366	187797+	protein of unknown function DUF523	10	15	25	25	96
CP000568	8811	8811 G	A	SYN	One	1648	R	Che0004	3874	9702+	YD repeat containing protein	12	14	26	27	96
CP000568	2476065	2476065-	A	SYN	Ins	320	K	Che2083	2475825	2477024+	DNA-directed DNA polymerase	15	11	26	27</	

CP000568	1273130	1273130	T	INTERGENIC	Ins							4	7	11	12	92
CP000568	1495972	1495972	T	SYN	Ins	1021 K	Che1235	1478377	1490034-	Cellulose 1,4-beta-cellulobiosidase		7	4	11	12	92
CP000568	1946531	1946531 T	C	NON	One	531 T	A	Che1616	1945599	phage minor structural protein		3	8	11	12	92
CP000568	780355	780355 C	T	SYN	One	20 P	P	Che0633	780296	780781+		6	6	12	13	92
CP000568	870718	870718 T	C	NON	One	560 Y	H	Che0714	869041	871125+	hydroxymethylbutenyl pyrophosphate reductase	6	6	12	13	92
CP000568	913540	913540 C	T	NON	One	155 R	C	Che1584	1913323	1914302-	two component transcriptional regulator, AraC	8	4	12	13	92
CP000568	2720003	2720003 T	-	NON	Del	21 N	-	Che2272	2701941	2702855+	conserved hypothetical protein	16	6	22	24	92
CP000568	2782553	2782553 G	A	NON	One	264 L	F	Che2334	2781498	2783342-	polysaccharide biosynthesis protein CapD	13	9	22	24	92
CP000568	321569	321569 G	A	NON	One	671 E	K	Che0260	319559	321682+	peptidase S1 and S6, chymotrypsin/Hap	15	9	24	26	92
CP000568	1205955	1205955 G	A	NON	One	187 E	K	Che1008	1205397	1206518+	aminodeoxychorismate lyase	12	8	20	22	91
CP000568	196295	196295 A	A	SYN	Ins	335 K	K	Che0156	195093	196934+	Radical SAM	8	13	23	21	89
CP000568	1627581	1627581 G	A	INTERGENIC	Ins							13	8	21	23	91
CP000568	2017515	2017515 C	C	INTERGENIC	Ins							13	8	21	23	91
CP000568	3665258	3665258 G	A	INTERGENIC	One							12	9	21	23	91
CP000568	2706499	2706499 A	T	INTERGENIC	Ins							12	18	30	33	91
CP000568	2800249	2800249 A	A	SYN	Ins	52 K	K	Che2427	2300096	2901802+	protein of unknown function DUF1385	16	14	30	33	91
CP000568	1403386	1403386 G	G	NON	One	170 T	A	Che1175	1402529	1403338+	hypothetical protein	3	9	10	10	89
CP000568	533461	533461 C	T	NON	One	704 P	L	Che0423	531351	533972+	iron-containing alcohol dehydrogenase	4	6	8	9	89
CP000568	3379326	3379326 T	C	SYN	One	379 S	S	Che2860	3378190	3379900+	hypothetical protein precursor	4	4	8	9	89
CP000568	244074	244074 -	A	SYN	Ins	15 Q	Q	Che0201	244031	244753+	glutamate synthase, alpha subunit-like	7	10	17	19	89
CP000568	1194190	1194190 C	T	NON	One	9 R	K	Che0996	1189887	1194215-	DNA polymerase III, alpha subunit	9	6	17	19	89
CP000568	3749144	3749144 G	A	SYN	One	777 V	V	Che3171	3746814	3749261+	S-layer domain-like	5	12	17	19	89
CP000568	97575	97575 C	C	INTERGENIC	One							8	7	15	17	88
CP000568	1330160	1330160 C	T	NON	Mul	254 G	R	Che1115	1329264	1330919-	Tn7-like transposition protein C	4	11	15	17	88
CP000568	3159478	3159478 -	T	SYN	Ins	57 K	K	Che2678	3159114	3159647-	hypothetical protein	8	7	15	17	88
CP000568	1248961	1248961 -	T	SYN	Ins	431 K	K	Che1046	1248913	1250253+	extracellular solute-binding protein, family 1	9	4	3	22	88
CP000568	8636341	8636341 -	T	NON	One	187 C	C	Che2207	8635725	8636900-	acyltransferase	10	13	23	26	88
CP000568	2791484	2791484 -	A	SYN	Ins	285 F	F	Che2341	2791172	2792338-	glycosyl transferase, family 2	13	10	23	26	88
CP000568	1103031	1103031 -	T	SYN	Ins	741 K	K	Che0918	1101624	1105253-	cellulosome enzyme, dockerin type I	4	3	7	8	87
CP000568	949261	949261 C	T	NON	One	42 H	Y	Che0785	949138	949440+	hypothetical protein	6	7	14	16	87
CP000568	3432750	3432750 A	A	INTERGENIC	Del							7	6	20	23	87
CP000568	863219	863219 A	A	SYN	Ins	66 K	K	Che0705	863024	863260+	hypothetical protein	10	11	18	21	87
CP000568	272547	272547 -	T	SYN	Ins	63 F	F	Che2291	2725445	272735-	hypothetical protein	8	13	21	24	87
CP000568	2320021	2320021 G	A	NON	One	43 S	F	Che1944	2319600	2320148-	protein of unknown function DUF458	12	14	26	30	87
CP000568	2382985	2382985 -	T	INTERGENIC	Ins							14	14	28	32	87
CP000568	3379300	3379300 T	G	NON	One	371 C	G	Che2860	3378190	3379900+	hypothetical protein precursor	3	3	6	7	86
CP000568	234760	234760 -	A	SYN	Ins	107 E	E	Che1885	223844	2239013+	phage integrase-like SAM-like	7	11	18	21	86
CP000568	2873484	2873484 -	A	NON	One	245 K	K	Che2407	2872750	2874374+	AAA ATPase, central region	7	11	18	21	86
CP000568	2872117	2872117 -	A	INTERGENIC	Ins							14	5	19	22	86
CP000568	138885	138885 C	T	SYN	One	328 P	P	Che0106	137902	139143+	GTP cyclohydrolase II	8	16	24	28	86
CP000568	269914	269914 -	T	INTERGENIC	Ins							6	11	17	20	85
CP000568	1020112	1020112 T	T	SYN	Ins	3 N	N	Che0841	1018890	1020119-	mutants block sporulation after engulfment	11	6	17	20	85
CP000568	2892757	2892757 -	T	SYN	Ins	278 K	K	Che2422	2892517	2893590-	hypothetical protein	5	11	16	19	84
CP000568	530864	530864 G	A	NON	One	60 G	S	Che0422	530687	531370+	CoA-binding	5	5	10	12	83
CP000568	3758418	3758418 -	A	INTERGENIC	Ins							7	11	18	22	82
CP000568	82364	82364 -	A	SYN	Ins	2324 E	E	Che0056	75394	89286+	Ig-like, group 2	7	6	13	16	81
CP000568	3607977	3607977 G	A	NON	One	590 K	K	Che0989	3506208	3508162+	glycosyltransferase 36	5	12	17	21	81
CP000568	2509967	2509967 G	A	INTERGENIC	One							9	23	22	27	81
CP000568	2152897	2152897 -	T	SYN	Ins	10 N	N	Che1817	2152557	2152925-	urease, beta subunit	7	7	14	18	78
CP000568	3613913	3613913 -	A	SYN	Ins	2 K	K	Che3073	3613910	3614581+	HAD-superfamily hydrolase, subfamily IA, variant	11	3	14	18	78
CP000568	3630169	3630172 ATCT	GTCA	NON	One	1647 P	-	Che3078	3625229	3632170+	cellulosome anchoring protein, cohesion region	2	1	3	4	75
CP000568	245709	245711 TCC	CTCT	NON	One	355 A	-	Che2691	2456901	2458295-	cellulosome anchoring protein, cohesion region	2	4	6	8	75
CP000568	2263439	2263439 T	-	NON	Del	86 K	-	Che1005	2262465	2263695+	glycosyl transferase, family 28	6	6	12	16	75
CP000568	1329105	1329118 TTGAAGGCAAGTCC	-	NON	Del	52 N	-	Che1114	1327372	1329258-	Tn7-like transposition protein D	3	5	8	11	73
CP000568	2245662	2245662 T	C	SYN	One	410 L	L	Che1890	2244759	2246891-	cellulosome enzyme, dockerin type I	4	1	5	7	71
CP000568	2245669	2245671 TCT	CCG	NON	One	408 E	-	Che1890	2244759	2246891-	cellulosome enzyme, dockerin type I	4	1	5	7	71
CP000568	1132396	1132396 C	T	SYN	Ins	49 G	G	Che0945	1132140	1133132-	metallophosphoesterase	5	7	15	17	71
CP000568	3065054	3065055 GA	TT	INTERGENIC	Mul							9	7	16	30	53
CP000568	3065049	3065051 GGT	TGC	NON	One	155 W	-	Che2592	3064586	3065053+	hypothetical protein	9	7	16	31	52
CP000568	542230	542230 T	C	INTERGENIC	One							2	4	6	12	50
CP000568	1476864	1476864 A	G	INTERGENIC	One							6	3	9	18	50
CP000568	3643837	3643837 C	T	NON	One	69 A	T	Che3089	3643649	3644041+	UspA	4	9	13	26	50
CP000568	2125777	2125777 C	T	NON	One	490 A	V	Che1798	2124309	2126225+	ABC transporter related	4	7	11	23	48
CP000568	290766	290766 -	TATA	INTERGENIC	Ins							2	3	5	11	45
CP000568	1083495	1083495 T	C	NON	Mul	295 E	G	Che0906	1083026	1084378-	Radical SAM	3	3	6	14	43
CP000568	2495816	2495818 CGA	-	NON	Del	158 G	-	Che2099	2495655	2496287-	nucleoside recognition	1	4	5	12	42
CP000568	2125349	2125349 C	-	SYN	Ins	347 R	R	Che1779	2125399	2126225-	ABC transporter related	5	4	8	19	42
CP000568	2874591	2874591 A	-	NON	One	41 A	A	Che2088	2874469	2875197+	phage shock protein A, PspA	5	4	9	22	41
CP000568	1903064	1903064 C	T	NON	One	719 E	K	Che0912	1908285	1902518-	glycoside hydrolase, family 10	4	2	6	15	40
CP000568	3342638	3342638 G	A	NON	Mul	565 E	K	Che2825	3340946	3342793+	conserved hypothetical protein precursor	8	7	15	39	38
CP000568	1851137	1851137 A	T	NON	One	306 Y	H	Che1525	1850184	1852052-	protein of unknown function DUF214	4	5	9	24	37
CP000568	3342659	3342659 A	G	SYN	Del	48 B	Q	Che2826	3340946	3342793+	conserved hypothetical protein precursor	8	7	15	30	37
CP000568	3342625	3342627 TGA	-	NON	Del	560 N	-	Che2825	3340946	3342793+	conserved hypothetical protein precursor	8	7	15	41	37
CP000568	1855366	1855366 A	G	NON	One	209 S	P	Che1529	1854740	1855992-	transposase	1	3	4	11	36
CP000568	509539	509539 -	T	SYN	Ins	6 K	K	Che0407	508249	509556-	Radical SAM	1	5	6	17	35
CP000568	3342604	3342621 GATAGGGTATAAACAGG	AAAGGGTGTAGATCAGGGGA	NON	One	553 L	-	Che2825	3340946	3342793+	conserved hypothetical protein precursor	7	6	13	37	35
CP000568	3342592	3342592 A	A	SYN	One	549 S	S	Che2825	3340946	3342793+	conserved hypothetical protein precursor	8	5	13	38	34
CP000568	2271533	2271535 GCC	-	NON	One	113 A	T	Che1990	2371523	2371870+	hypothetical protein	2	2	4	3	9
CP000568	790216	790216 G	A	SYN	One	233 S	S	Che0639	789712	7900154-	glycoside hydrolase, family 13-like	1	3	4	12	33
CP000568	3327878	3327881 ATTT	TTAA	INTERGENIC	One							4	3	7	21	33
CP000568	3336374	3336374 A	C	NON	One	66 T	A	Che2821	3336179	3337273+	response regulator receiver modulated CheB	4	2	6	19	32
CP000568	980941	980941 T	C	INTERGENIC	One							4	5	9	28	32
CP000568	619235	619242 CTTTTTTC	TCITTTTTT	NON	One	214 S	-	Che0507	619228	619875-	Exonuclease, RNase T and DNA polymerase III	2	2	4	13	41
CP000568	1627485	1627529 TTGTGCGAATAATTATTGTGCGAATAATTATTGTGCGAATAATA	-	INTERGENIC	Del							3	2	5	16	31
CP000568	2282632	2282632 G	A	NON	One	113 A	V	Che1915	2281578	2282969-	periplasmic sensor signal transduction histidine	1	9	10	32	31
CP000568	2100481	2100481 C	T	NON	One	173 A	T	Che1778	2100038	210099						

CP000568	371791	371791 A	G	NON	One	322 Y	C	Ctbe0296	370827	372302+	hypothetical protein	1	2	3	18	17
CP000568	2412393	2412393 G	A	NON	Mul	984 P	L	Ctbe2032	2409329	2415343-	hypothetical protein	1	2	3	18	17
CP000568	445662	445662 C	T	NON	One	110 T	I	Ctbe0355	445334	446512+	conserved hypothetical protein	1	3	4	24	17
CP000568	2600988	2600988 G	AGT	NON	One	515 A	-	Ctbe2182	2599446	2602235+	Ig-like, group 2	1	2	3	19	16
CP000568	3144485	3144487 TAA	GGAACCG	NON	One	361 L	-	Ctbe2664	3143384	3145105+	ABC-1	1	2	3	19	16
CP000568	614571	614571 C	-	INTERGENIC	Del							1	2	3	21	14
CP000568	2127018	2127020 GCA	C	NON	One	201 A	P	Ctbe1800	2128418	2127929+	Peptidoglycan-binding LysM	1	2	3	22	14
CP000568	3310989	3310989 T	C	SYN	One	99 R	R	Ctbe2809	3310693	3314658+	glycoside hydrolase, family 16	1	2	3	23	13
CP000568	124029	124029 C	T	SYN	One	11 S	S	Ctbe0091	123997	126126+	Peptidoglycan glycosyltransferase	1	2	3	24	12
CP000568	1958491	1958491 G	A	STOP	One	435 R	*	Ctbe1631	1958009	1959793-	conserved hypothetical protein	2	1	3	25	12
CP000568	3007869	3007869 A	G	NON	One	482 N	D	Ctbe2537	3006426	3008225+	sulfate adenylyltransferase, large subunit	1	2	3	24	12
CP000568	3016361	3016362 TG	-	NON	Del	110 F	-	Ctbe2548	3016032	3017543+	Alpha-N-arabinofuranosidase	1	2	3	25	12
CP000568	1260236	1260236-	T	SYN	Ins	12 Y	Y	Ctbe1055	1260203	1261411+	protein of unknown function DUF58	1	2	3	27	11
CP000568	2397277	2397277 A	G	SYN	One	140 L	L	Ctbe2018	2396459	2397694-	conserved hypothetical protein	1	3	4	40	10

2397277

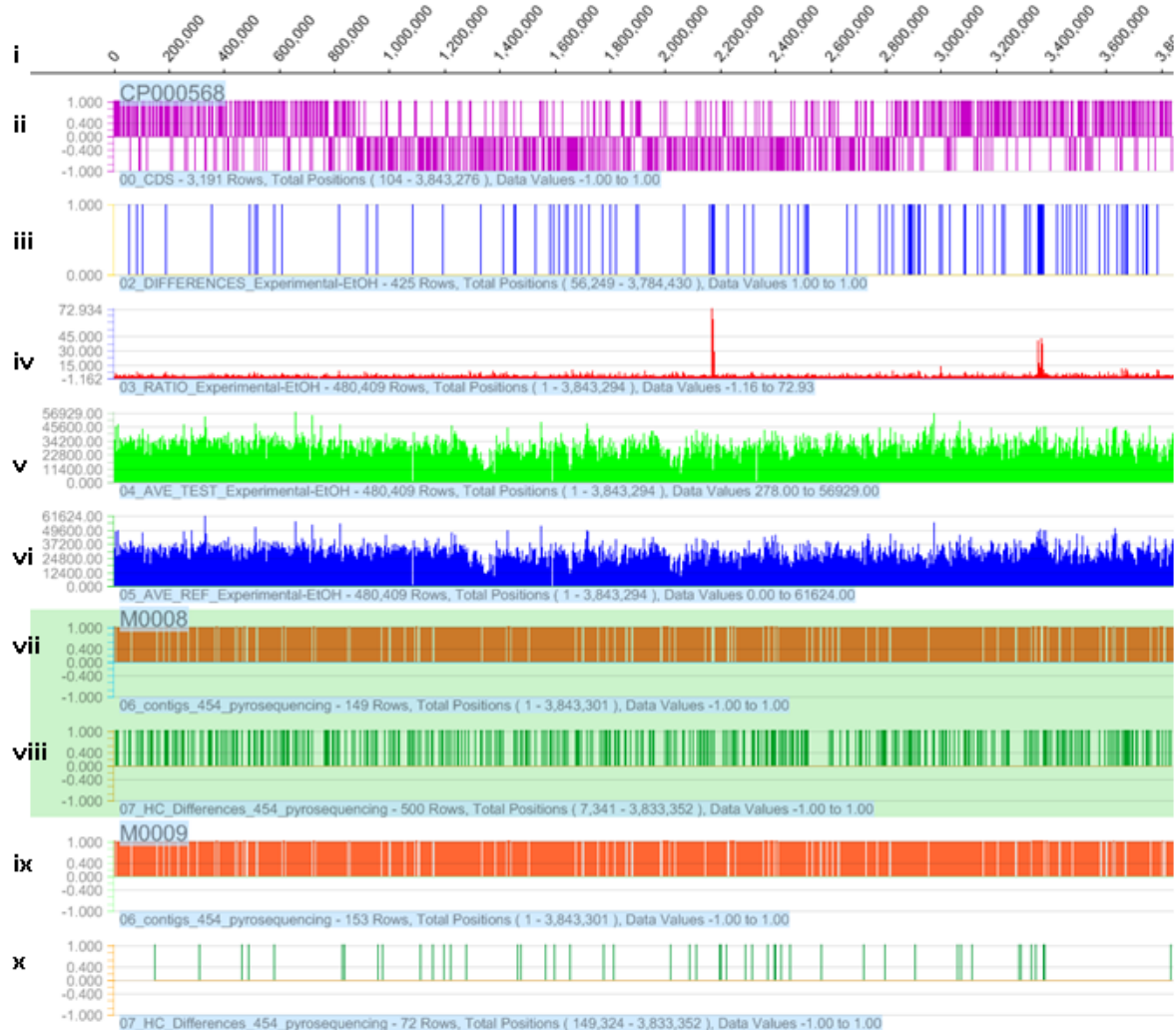


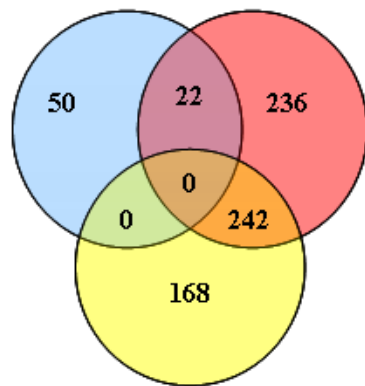
Figure S1. Summary of pyrosequencing and comparative genome sequencing (CGS) resequencing data for *C. thermocellum* wild-type strain 27405 and the EA mutant strain. Genome positions (i) and genes (ii) are indicated. 425 differences for mutant strain EA were identified by CGS (iii), along with two putative regions where DNA was deleted (iv). CGS microarray signal intensities are represented for the mutant (v) and wild-type (vi) strains. Contiguous regions of DNA that mapped to wild-type reference genome from pyrosequencing data for the mutant (153) and strain 27405 (149) are shown, vii and ix respectively. 500 putative high-confidence differences were determined by pyrosequencing for mutant strain EA (viii) and 72 putative high-confidence differences were identified for strain 27405 (x).

All HCDiff wild-type
pyrosequencing
differences

All HCDiff EA
mutant
pyrosequencing
differences

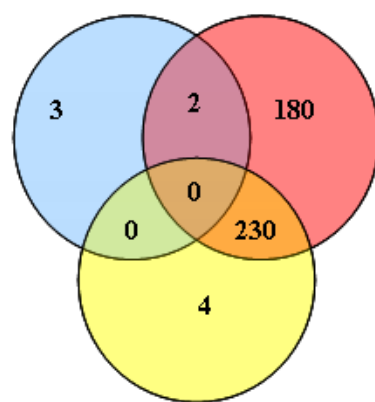
Filtered wild-type
pyrosequencing
differences

Filtered EA mutant
pyrosequencing
differences



All EA mutant CGS
differences

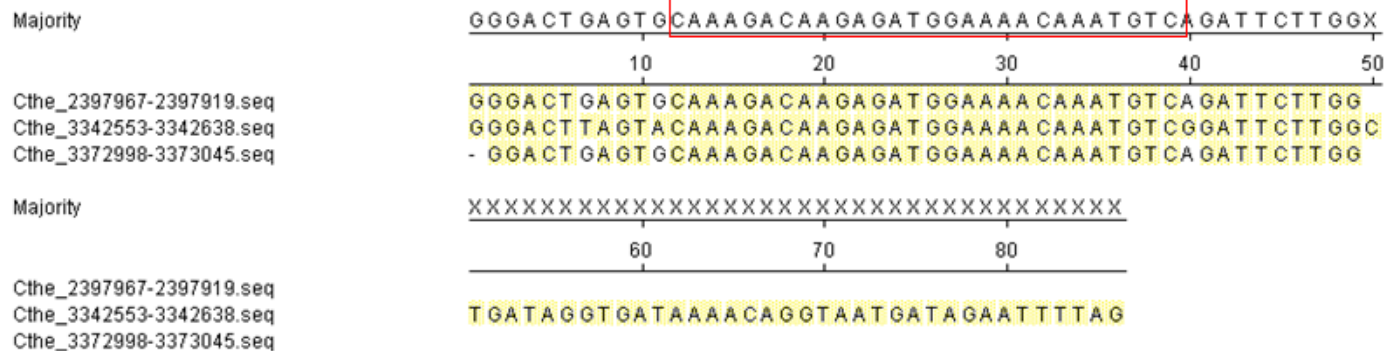
A



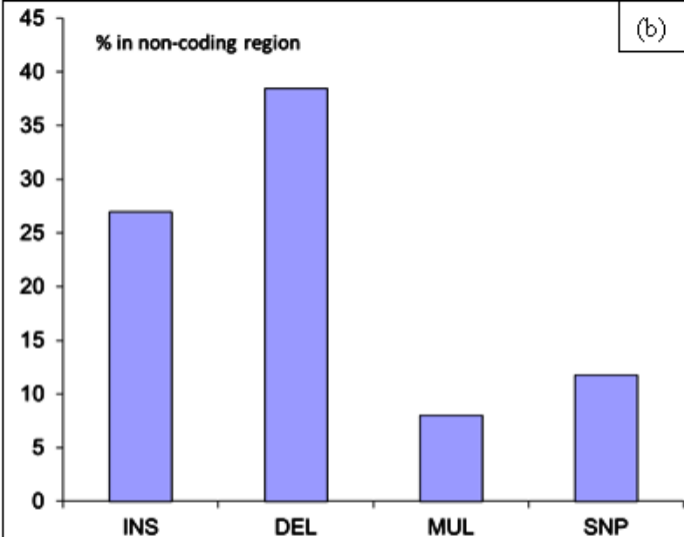
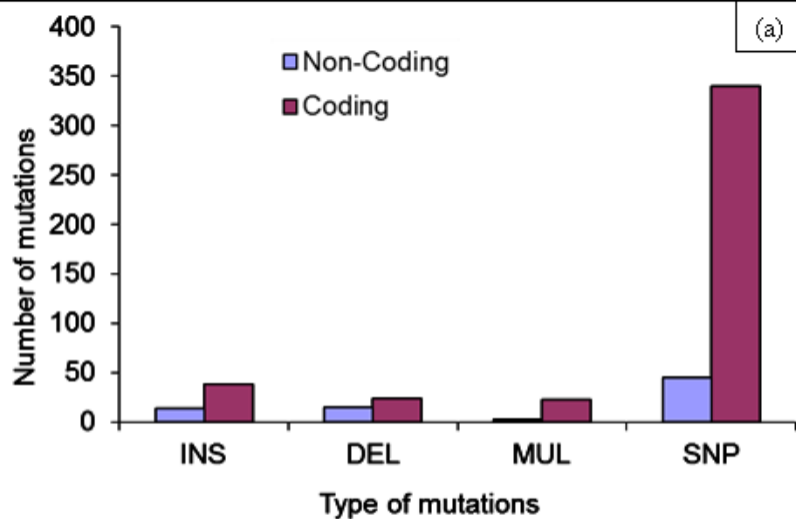
Filtered EA mutant CGS
differences

B

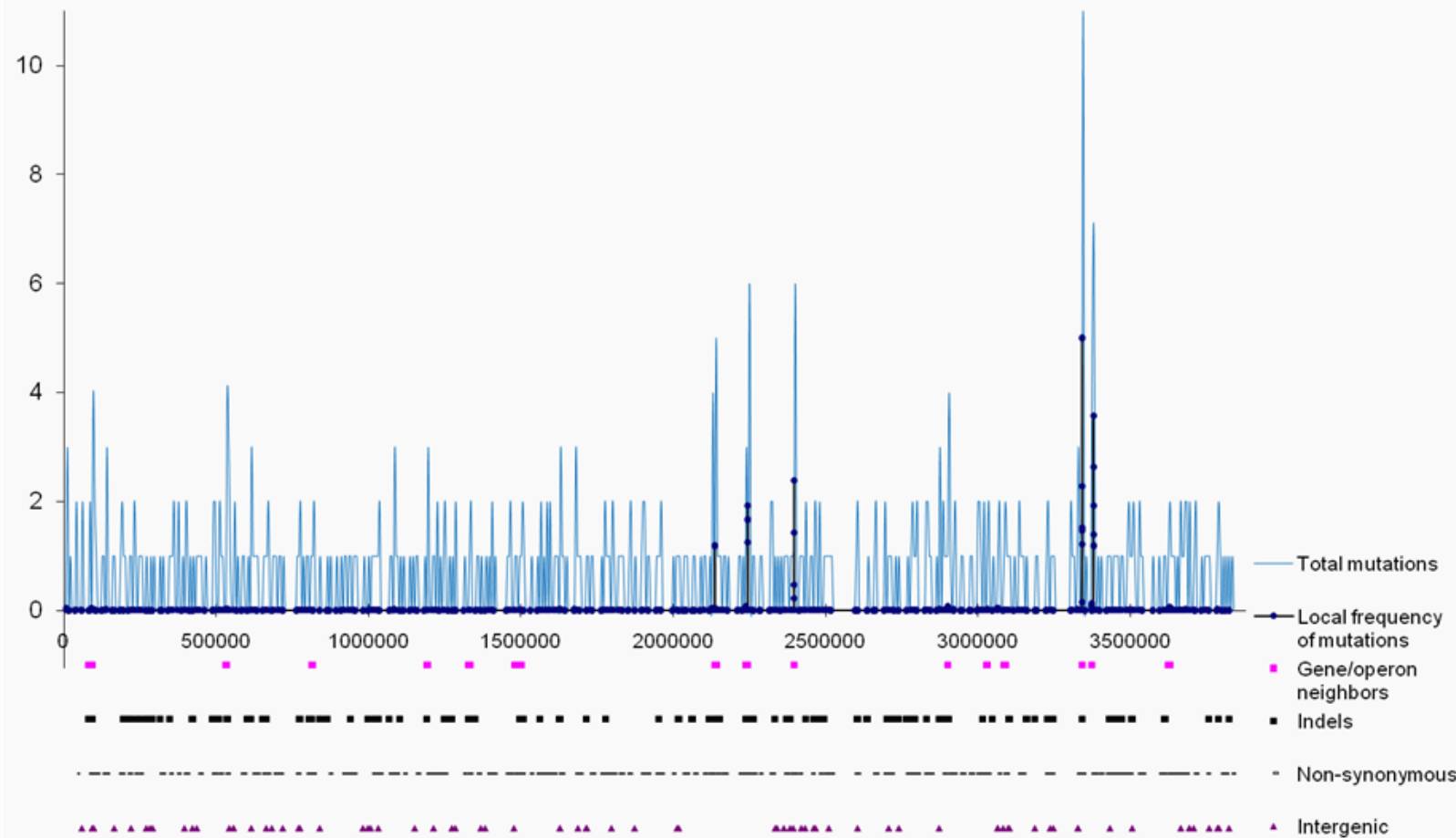
Supplemental Figure S2. Venn analysis of pyrosequencing and CGS resequencing data. 'High confidence' genetic differences identified by pyrosequencing for wild-type and mutant EA strains and all putative CGS differences are shown (A). Pyrosequencing differences with 'variation values' less than 80% and CGS differences defined as either 'low' or 'medium' confidence were filtered out during this analysis (B). All genetic differences and descriptions are shown in Tables Supplemental S1-3.



Supplemental Figure S3. Repetitive DNA detected in “High Confidence Differences (HCDiffs)” regions with low variation values. Twenty five putative HCDiffs were reported by the GSMapper software for conserved hypothetical genes Cthe_2018, Cthe_2825-Cthe_2826, and Cthe_2854-Cthe_2855 for the wild-type strain and the majority were also reported in the mutant strain. A closer examination revealed low variation values for the putative differences (mean of 30) and a CLUSTALW alignment using MegAlign (DNASTar 8.0.2) revealed 28 nucleotides were repeated (red box). A previous study indicated so called HCDiffs with low variation values should be interpreted with caution and are likely false positives. (S. Yang, M. L. Land, D. M. Klingeman, D. A. Pelletier, T.-Y. S. Lu, S. L. Martin, H.-B. Guo, J. C. Smith, S. D. Brown. Paradigm for industrial strain improvement identifies sodium acetate tolerance loci in *Zymomonas mobilis* and *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences*, 2010; 107 (23): 10395 DOI: [10.1073/pnas.0914506107](https://doi.org/10.1073/pnas.0914506107)).



Supplemental Figure S4. Distribution analysis of EA mutant pyrosequencing data. 16 mutational hotspots identified in this analysis are described in detail in Table 3.



Supplemental Figure S5. Types of genomic mutations identified in the EA mutant by pryosequencing. Types and numbers of identified mutations in coding and non-coding regions of the EA genome (a) and percentage of different types of mutations found in non-coding regions of the genome (b).

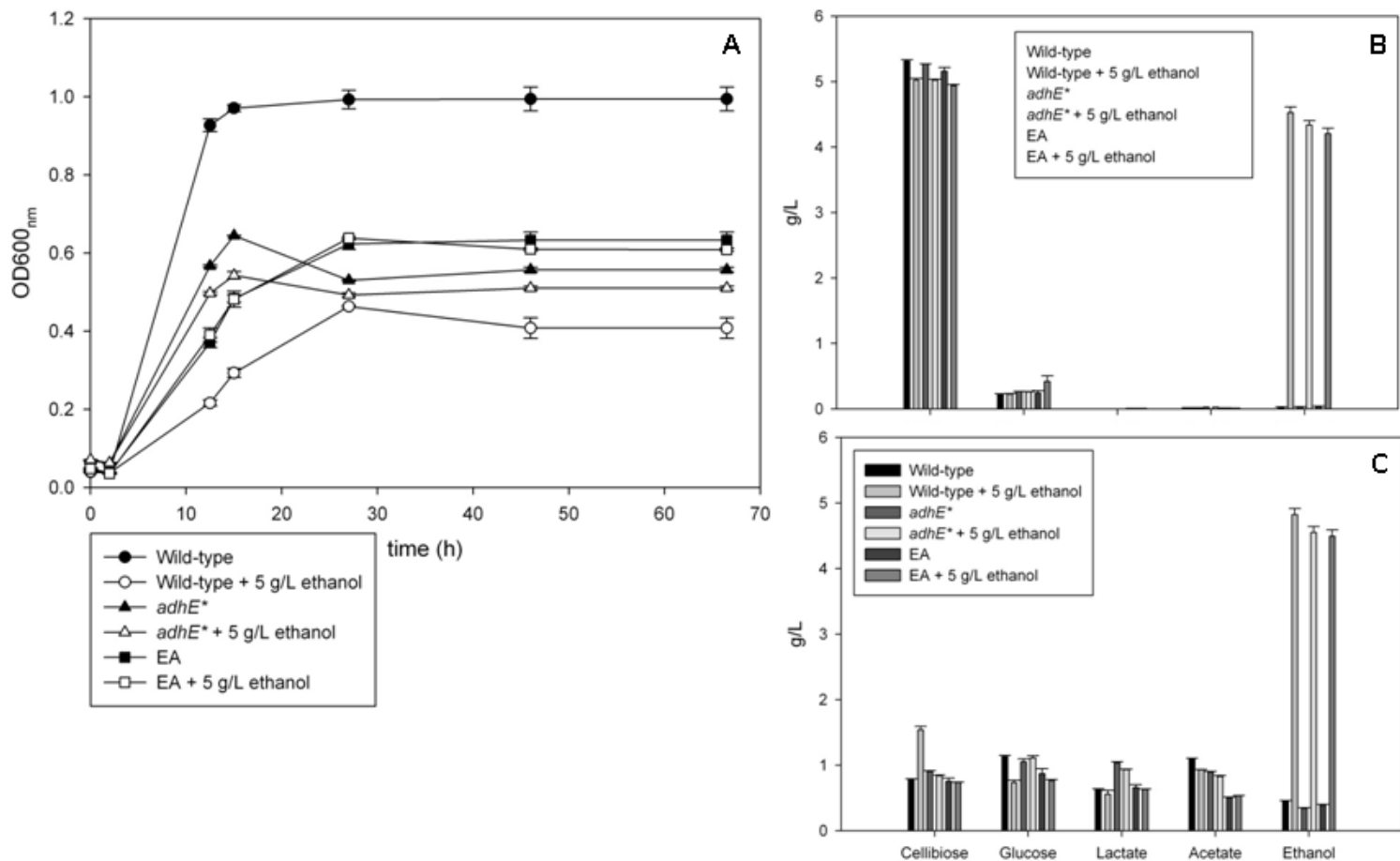


Figure S6. Growth and extracellular metabolite analysis of *C. thermocellum* mutants *adhE, EA and wild-type 27405.** All cultures (including inocula) were grown in media for thermophilic clostridia (MTC) containing 5 g/L cellobiose and 2 g/L yeast extract at 55°C and 125 rpm. Anaerobic ethanol was added at a concentration of 5 g/L to select cultures. A 1 mL inoculum from a serum bottle was added to each Balch tube containing 10 mL of medium. (A) Culture growth was monitored by measuring optical densities at 600 nm. Samples were taken for HPLC analysis at the beginning (B) and end (C) of the experiment. For this purpose, samples were filtered through 0.2 μ m acrodiscs and acidified with sulfuric acid. Metabolite analysis was performed using a LaChrom Elite system (Hitachi High Technologies America, Inc.) equipped with a refractive index detector (Model L-2490). Metabolites (cellobiose, glucose, lactic acid, acetic acid and ethanol) were separated at a flow rate of 0.5 ml/min in 5 mM H₂SO₄ using an Aminex HPX- 87H column (Bio-Rad Laboratories, Inc.).

Added Ethanol (v/v)

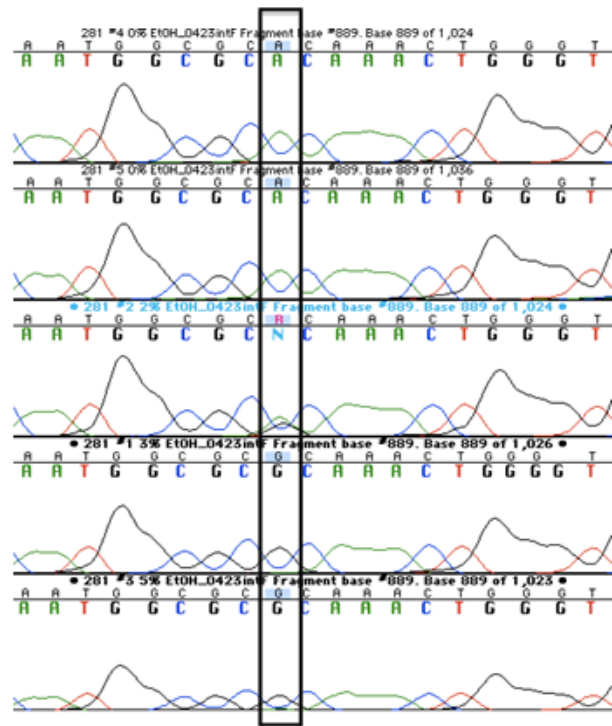
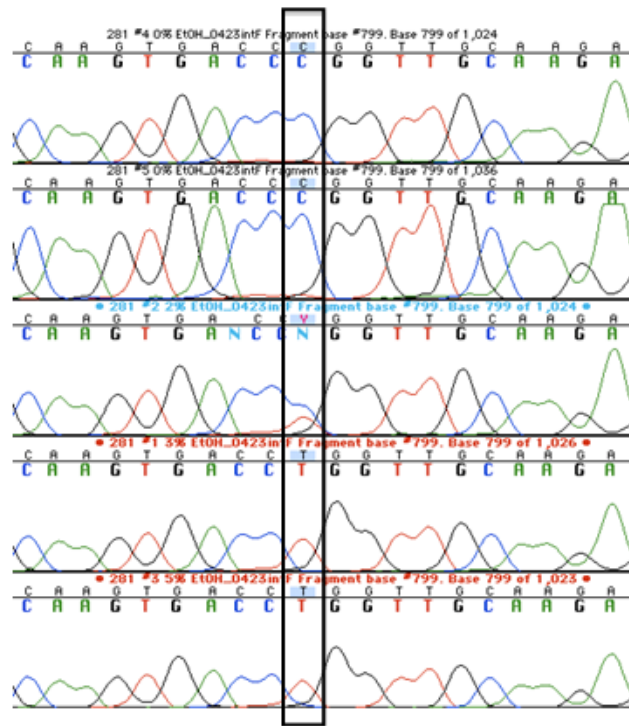
0%

1%

2%

3%

5%



Supplemental Figure S7. Chromosomal wild-type *adhE* gene replacement to *adhE version observed with increasing ethanol concentrations.** Sanger data for SNPs from PCR products amplified using flanking chromosomal primers.