Supplementary Information for

Long-term adaptive evolution of Shewanella oneidensis MR-1 for establishment of high concentration Cr(VI) tolerance

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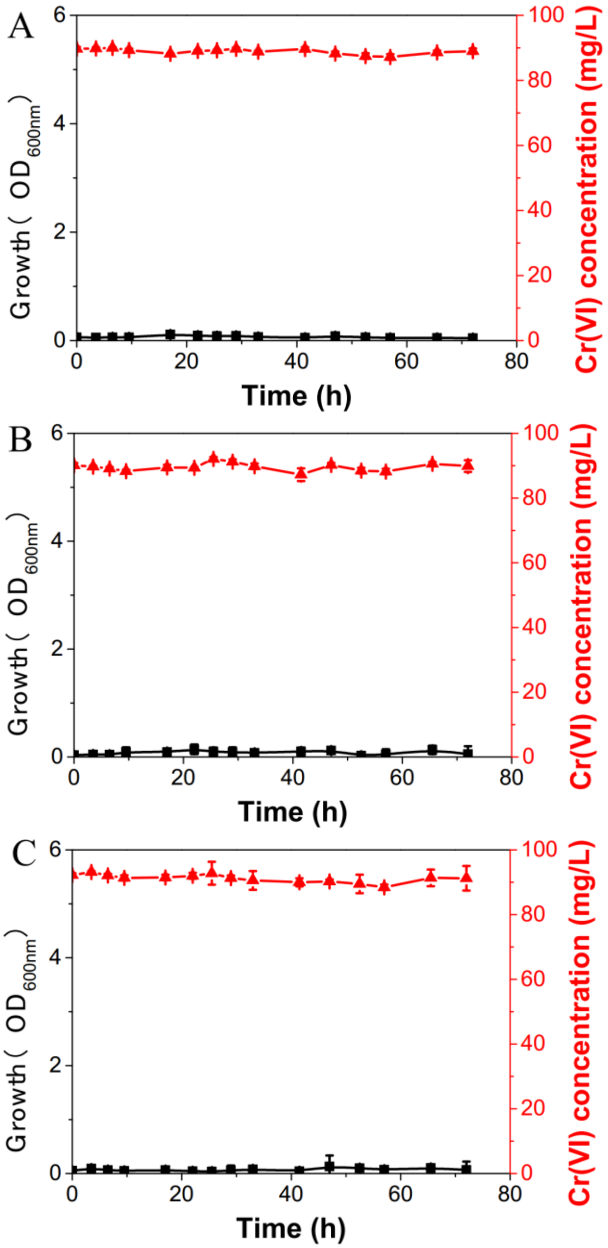

Figure S1. Growth rate (black line) and reduction kinetics (red line) of adding initial concentration of 104mg/L Cr(VI) into different period evolved-LB forms of *Shewanella oneidensis* MR-1. (A) Evolved strain for 40 days (population D40) was inoculated in LB broth. (B) Evolved strain for 80 days (population D80) was inoculated in LB broth. (C) Evolved strain for 120 days (population D120) was inoculated to LB broth.

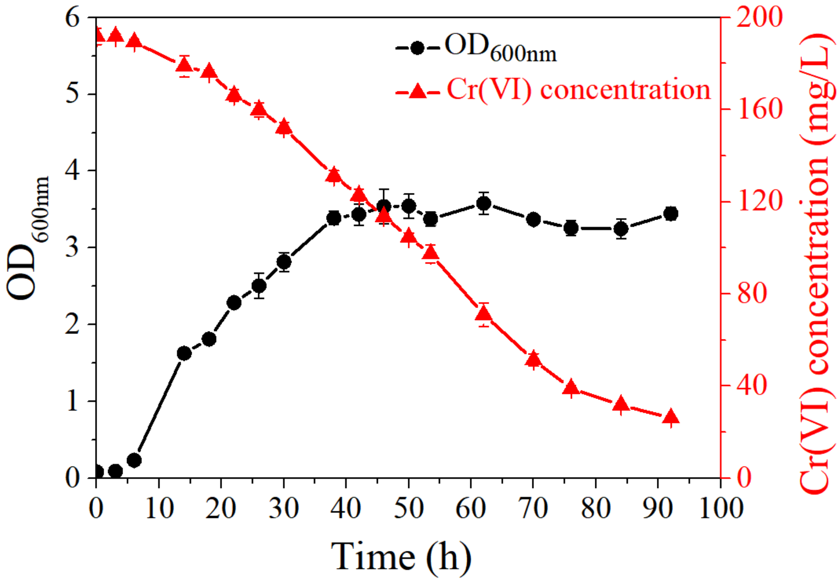

Figure S2. Growth rate (black line) and reduction kinetics (red line) of adding initial concentration of 190 mg/L Cr(VI) into double nutrients LB medium (10 g/L yeast extract, 10 g/L NaCl and 20 g/L tryptone) in population D120Cr.

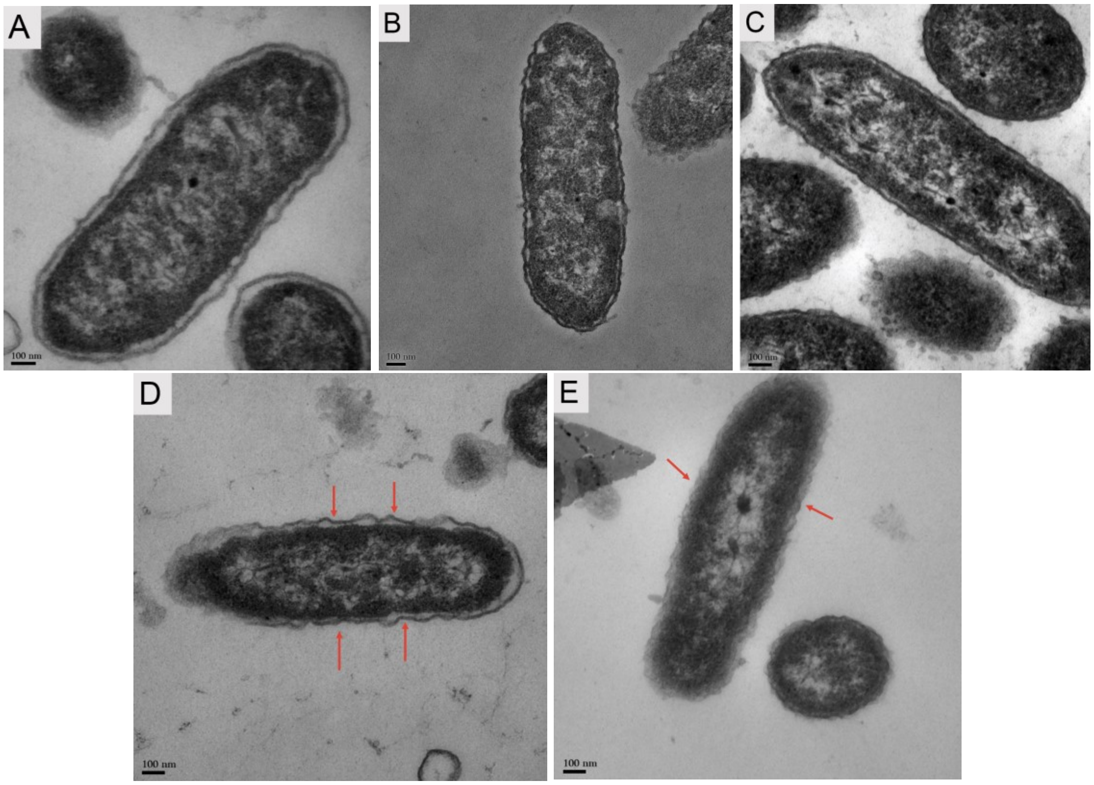

Figure S3. TEM images in different evolved forms. (A) The original strain D0. (B) Population D80 cultured for 80 days in LB medium. (C) Population D120 cultured for 120 days in LB medium. (D) Population D80Cr cultured for 80 days in LB+Cr(VI) medium. (E) Population D120Cr cultured for 120 days in LB+Cr(VI) medium.

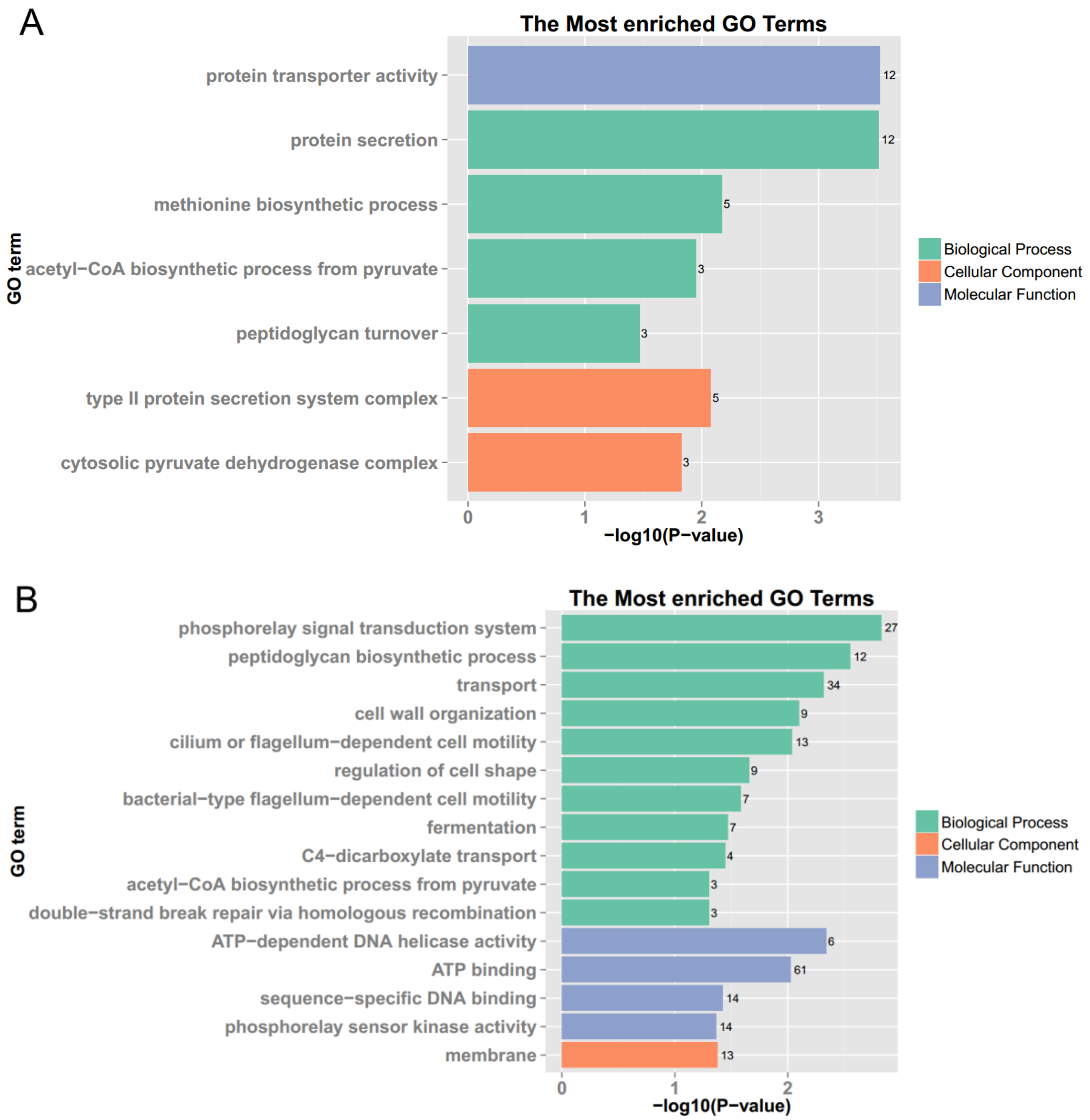

Figure S4. The most enriched GO Terms of mutation genes in population D40Cr (A) and D80Cr (B). The P_value from the result of enrichment analysis is the X-axis, Y-axis is about GO terms which including three categories, biological process, cellular component and molecular function.

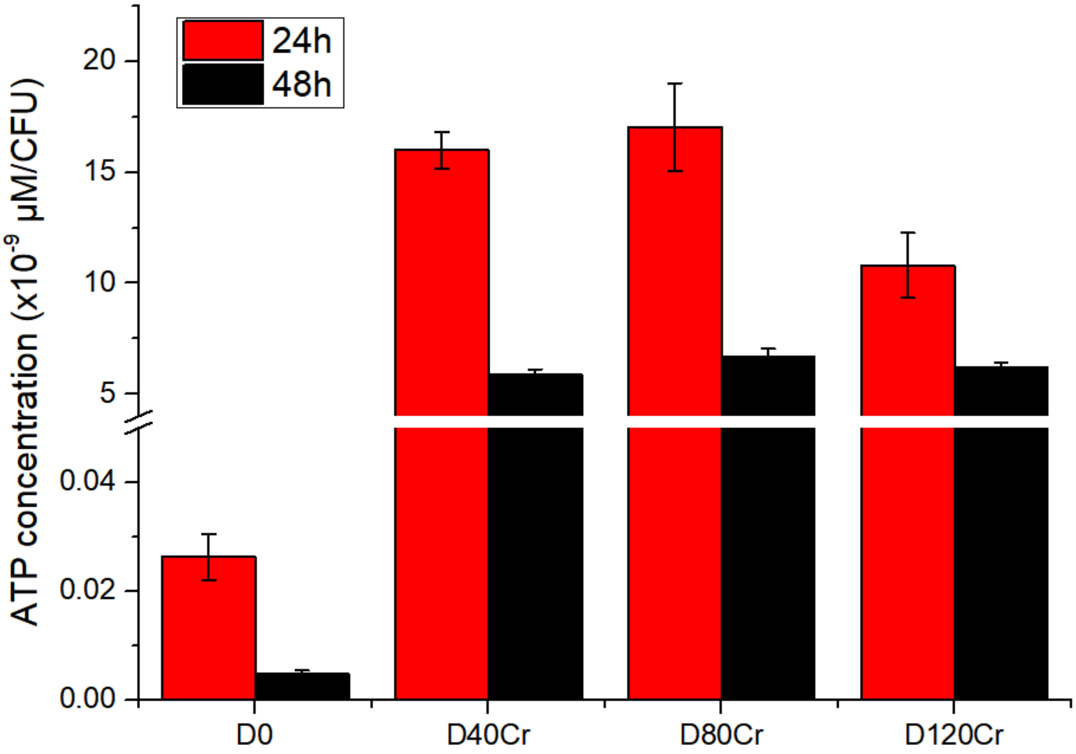

Figure S5. ATP concentration in evolved strains of populations D0, D40Cr, D80Cr and D120Cr at growth time 24 and 48 h, respectively. The red bar is cultured for 24h and the black bar is 48h.

Table S1 Incubation time and increase of Cr(VI) concentration

Time (Day)	Concentration of Cr(VI) (mg/L)	Time (Day)	Concentration of Cr(VI) (mg/L)
2	9.36	62	133.12
4	9.36	64	133.12
6	9.36	66	133.12
8	23.4	68	133.12
10	23.4	70	133.12
12	23.4	72	149.76
14	37.44	74	149.76
16	46.8	76	149.76
18	46.8	78	149.76
20	46.8	80	149.76
22	65.52	82	166.4
24	65.52	84	166.4
26	65.52	86	166.4
28	65.52	88	166.4
30	65.52	90	166.4
32	65.52	92	167.44
34	93.6	94	167.44
36	93.6	96	167.44
38	93.6	98	167.44
40	93.6	100	167.44
42	93.6	102	182
44	99.84	104	182
46	99.84	106	182
48	99.84	108	182
50	99.84	110	182
52	116.48	112	193.44
54	116.48	114	193.44
56	116.48	116	193.44
58	116.48	118	193.44
60	116.48	120	193.44

Table S2 List of mutations occurred in population D40.

Locus tag	Gene name	Reference genome position	Reference base	Base change	Mutation class	Mutation region	Amino acid change
Group A							
SO_0719	SO_0719	735021	C	A	missense_variant	protein_coding	p.Ser542Ile
SO_3130	gltX	3260663	T	G	missense_variant	protein_coding	p.His178Pro
SO_3337	SO_3337	3489946	A	C	upstream_gene_variant		

Table S3 List of mutations occurred in population D120.

Locus tag	Gene name	Reference genome position	Reference base	Base change	Mutation class	Mutation region	Amino acid change
Group A							
SO_0012	SO_0012	18204	C	T	upstream_gene_variant		
SO_4091	tldD	4246371	C	T	missense_variant	protein_coding	p.Met285Ile
Group B							
SO_2671	lys	2799393	T	C	synonymous_variant	protein_coding	p.Leu158Leu
Group C							
SO_0821	macB	837053	G	A	missense_variant	protein_coding	p.Ala288Thr

Table S4 List of mutations occurred in population D40Cr.

Locus tag	Gene name	Reference genome position	Reference base	Base change	Mutation class	Mutation region	Amino acid change
Group A							
SO_0092	deoD	100886	C	T	missense_variant	protein_coding	p.Arg25Lys
SO_r008	rrlC	271458	A	G	upstream_gene_variant	rRNA	
SO_0449	SO_0449	476335	C	T	stop_gained	protein_coding	p.Gln243*
SO_0809	azu	821593	G	A	missense_variant	protein_coding	p.Thr12Ile
SO_0821	macB	837053	G	A	missense_variant	protein_coding	p.Ala288Thr
SO_1030	metH	1070260	G	A	missense_variant	protein_coding	p.Ala93Val
SO_1602	pfaA	1685457	C	A	missense_variant	protein_coding	p.Ala236Ser
SO_1602	pfaA	1688726	G	A	upstream_gene_variant		
SO_4795	SO_4795	3124610	T	C	missense_variant	protein_coding	p.Leu11Pro
SO_3313	rodZ	3465177	T	C	missense_variant	protein_coding	p.Thr302Ala
SO_3506	nagB	3663222	C	T	upstream_gene_variant		
SO_3731	SO_3731	3874406	C	T	stop_gained	protein_coding	p.Gln69*
SO_4097	mreC	4255854	T	C	synonymous_variant	protein_coding	p.Gln80Gln
SO_4131	SO_4131	4286044	C	T	upstream_gene_variant		

SO_4149	SO_4149	4319628	C	T	missense_variant	protein_coding	p.Arg3340Trp
SO_4224	murE	4404350	A	G	upstream_gene_variant		
SO_4487	SO_4487	4674381	C	T	synonymous_variant	protein_coding	p.Leu63Leu
SO_4615	senC	4809541	A	G	synonymous_variant	protein_coding	p.Gln175Gln
Group B							
SO_1184	pth	1227963	C	G	synonymous_variant	protein_coding	p.Ala155Ala
SO_1311	SO_1311	1367115	T	G	synonymous_variant	protein_coding	p.Thr244Thr
Group C							
SO_0054	yhiN	60857	A	G	synonymous_variant	protein_coding	p.Leu301Leu
SO_0137	moeB	143935	C	T	missense_variant	protein_coding	p.Val70Ile
SO_0174	gspL	181023	T	C	missense_variant	protein_coding	p.Met197Thr
SO_0417	pilA	438321	T	C	upstream_gene_variant		
SO_0802	SO_0802	815411	C	T	synonymous_variant	protein_coding	p.Arg240Arg
SO_1126	dnaK	1169253	T	C	missense_variant	protein_coding	p.Ile168Thr
SO_4062	phsA	4216217	T	C	missense_variant	protein_coding	p.Ser512Gly
SO_4323	SO_4323	4511398	T	C	synonymous_variant	protein_coding	p.Ser497Ser

Table S5 List of mutations occurred in population D80Cr.

Locus tag	Gene name	Reference genome position	Reference base	Base change	Mutation class	Mutation region	Amino acid change
Group A							
SO_0311	ygiQ	317245	G	A	missense_variant	protein_coding	p.Gly757Asp
SO_0855	attH	881060	A	G	upstream_gene_variant		
SO_1372	SO_1372	1429913	C	T	missense_variant	protein_coding	p.Glu81Lys
SO_1724	pstA	1811923	G	A	missense_variant	protein_coding	p.Val409Ile
SO_1769	SO_1769	1853031	C	T	upstream_gene_variant		
SO_2561	SO_2561	2690998	C	A	stop_gained	protein_coding	p.Glu196*
SO_2633	trmU	2768917	T	C	missense_variant	protein_coding	p.Val66Ala
SO_2715	SO_2715	2834249	G	A	synonymous_variant	protein_coding	p.Gly186Gly
SO_3138	dctD	3267508	G	A	upstream_gene_variant		
SO_3483	SO_3483	3630912	C	T	synonymous_variant	protein_coding	p.Gly327Gly
SO_3611	SO_3611	3772444	T	C	synonymous_variant	protein_coding	p.Gln425Gln
SO_3748	ybiS	3895761	T	C	missense_variant	protein_coding	p.Leu182Pro
SO_4005	SO_4005	4147647	C	T	missense_variant	protein_coding	p.Ala51Thr

SO_4007	SO_4007	4149675	T	C	synonymous_variant	protein_coding	p.Leu12Leu
SO_4222	mraY	4396784	C	T	synonymous_variant	protein_coding	p.Lys358Lys
SO_4377	SO_4377	4572251	G	A	missense_variant	protein_coding	p.Gly497Asp
SO_4428	SO_4428	4619593	A	G	missense_variant	protein_coding	p.Ile77Thr
SO_4618	SO_4618	4813216	C	T	missense_variant	protein_coding	p.Pro334Ser
SO_4645	SO_4645	4842096	T	G	missense_variant	protein_coding	p.Glu40Ala
Group B							
SO_0308	SO_0308	313497	A	G	missense_variant	protein_coding	p.Phe238Leu
SO_0630	nosA	657471	T	C	synonymous_variant	protein_coding	p.Ser537Ser
SO_0680	SO_0680	698256	C	G	missense_variant	protein_coding	p.His546Asp
SO_0798	SO_0798	816182	G	A	upstream_gene_variant		
SO_1218	deoA	1265685	A	T	missense_variant	protein_coding	p.Ile312Phe
SO_1411	SO_1411	1467863	A	C	missense_variant	protein_coding	p.Trp227Gly
SO_2234	SO_2234	2351433	G	A	upstream_gene_variant		
SO_3092	SO_3092	3217017	C	A	missense_variant	protein_coding	p.Ala164Asp
SO_3232	flrA	3371945	T	C	missense_variant	protein_coding	p.Asn285Asp

SO_3492	mexF	3642994	A	G	missense_variant	protein_coding	p.Val40Ala
SO_3791	SO_3791	3943206	G	A	missense_variant	protein_coding	p.Gly311Ser
SO_4702	gor	4904192	T	C	missense_variant	protein_coding	p.Tyr153His
Group C							
SO_0126	SO_0126	133685	A	G	synonymous_variant	protein_coding	p.Arg209Arg
SO_0137	moeB	143935	C	T	missense_variant	protein_coding	p.Val70Ile
SO_0174	gspL	181023	T	C	missense_variant	protein_coding	p.Met197Thr
SO_0471	SO_0471	496053	G	A	missense_variant	protein_coding	p.Ala174Thr
SO_0546	rimK	569404	C	T	missense_variant	protein_coding	p.Thr158Ile
SO_0578	SO_0578	603064	T	C	upstream_gene_variant		
SO_1411	SO_1411	1468505	A	G	missense_variant	protein_coding	p.Phe13Leu
SO_1780	mtrF	1865610	T	C	missense_variant	protein_coding	p.Ile153Val
SO_2020	gsk	2119821	A	G	missense_variant	protein_coding	p.Asn132Ser
SO_2258	lolD	2373978	G	A	synonymous_variant	protein_coding	p.Val182Val
SO_2473	SO_2473	2595628	T	C	synonymous_variant	protein_coding	p.Ser193Ser
SO_2753	SO_2753	2873721	G	A	missense_variant	protein_coding	p.Ala572Val

SO_3130	gltX	3260663	T	G	missense_variant	protein_coding	p.His178Pro
SO_3504	nagX	3655325	T	G	synonymous_variant	protein_coding	p.Ile359Ile
SO_3855	sfcA	4001375	A	G	missense_variant	protein_coding	p.Asp193Gly
SO_4047	SO_4047	4201739	C	T	synonymous_variant	protein_coding	p.Leu32Leu
SO_4150	SO_4150	4325377	T	C	missense_variant	protein_coding	p.Phe157Ser
SO_4241	recQ	4423095	G	A	upstream_gene_variant		
SO_4657	SO_4657	4853822	A	G	missense_variant	protein_coding	p.Ile736Thr

Table S6 List of mutations occurred in population D120Cr

Locus tag	Gene name	Reference genome position	Reference base	Base change	Mutation class	Mutation region	Amino acid change
Group A							
SO_0038	hemF	43039	C	T	synonymous_variant	protein_coding	p.Leu296Leu
SO_0040	aroE	44289	G	A	synonymous_variant	protein_coding	p.Thr191Thr
SO_0048	SO_0048	55157	A	G	missense_variant	protein_coding	p.Tyr145His
SO_0071	SO_0071	83997	C	T	synonymous_variant	protein_coding	p.Gln88Gln
SO_0101	fdnG	110897	C	T	missense_variant	protein_coding	p.Ala732Val
SO_0151	SO_0151	159502	T	C	synonymous_variant	protein_coding	p.Leu19Leu
SO_0184	SO_0184	188649	C	T	missense_variant	protein_coding	p.Ala82Val
SO_0189	SO_0189	197007	C	T	missense_variant	protein_coding	p.Ala1507Val
SO_0324	ygbA	330468	C	T	synonymous_variant	protein_coding	p.Glu14Glu
SO_0341	SO_0341	343717	T	C	missense_variant	protein_coding	p.Asn764Ser
SO_0383	hsdM	398424	A	G	missense_variant	protein_coding	p.Leu233Pro
SO_0467	uvrD	492479	A	G	missense_variant	protein_coding	p.Thr568Ala

SO_0485	nosL	508915	A	G	missense_variant	protein_coding	p.His35Arg
SO_0666	gpE	683582	C	T	missense_variant	protein_coding	p.Ala15Val
SO_0702	SO_0702	720197	A	G	synonymous_variant	protein_coding	p.Gly82Gly
SO_0754	atmA	769746	T	C	missense_variant	protein_coding	p.Thr481Ala
SO_0812	SO_0812	824046	T	C	missense_variant	protein_coding	p.Leu53Pro
SO_0822	macC	838336	A	C	missense_variant	protein_coding	p.Gln57Pro
SO_0856	attFG	879614	C	T	missense_variant	protein_coding	p.Gly92Asp
SO_0908	SO_0908	936095	A	G	upstream_gene_variant		
SO_1034	btuC	1073664	A	G	missense_variant	protein_coding	p.Ser235Gly
SO_1150	rpiA	1194346	C	T	missense_variant	protein_coding	p.Asp153Asn
SO_1298	gspB	1349504	A	G	missense_variant	protein_coding	p.Glu231Gly
SO_1396	SO_1396	1458568	C	T	upstream_gene_variant		
SO_1429	dmsA	1492659	A	G	missense_variant	protein_coding	p.Asn490Ser
SO_1450	SO_1450	1510118	T	C	missense_variant	protein_coding	p.Asn56Ser
SO_1461	SO_1461	1520827	G	A	missense_variant	protein_coding	p.Asp386Asn

SO_1644	dnaE	1727667	A	G	missense_variant	protein_coding	p.Tyr453Cys
SO_1780	mtrF	1865985	C	T	missense_variant	protein_coding	p.Asp28Asn
SO_1781	mtrE	1867528	G	A	missense_variant	protein_coding	p.Ala238Val
SO_2308	SO_2308	2420926	C	T	synonymous_variant	protein_coding	p.Asp132Asp
SO_2381	SO_2381	2489613	G	A	upstream_gene_variant		
SO_2418	tnpA	2525578	A	G	synonymous_variant	protein_coding	p.Glu150Glu
SO_2487	edd	2610372	T	C	missense_variant	protein_coding	p.Thr9Ala
SO_2674	com	2800401	C	T	stop_gained	protein_coding	p.Gln13*
SO_2767	asnB	2890655	T	C	missense_variant	protein_coding	p.Val445Ala
SO_3279	SO_3279	3419927	A	T	missense_variant	protein_coding	p.Leu322Phe
SO_3294	xseA	3440018	G	A	synonymous_variant	protein_coding	p.Ser351Ser
SO_3483	SO_3483	3630597	T	C	synonymous_variant	protein_coding	p.Arg222Arg
SO_3534	murJ	3691134	A	G	missense_variant	protein_coding	p.Leu33Ser
SO_3542	xfp	3697539	C	T	missense_variant	protein_coding	p.Ala345Val
SO_3559	gshA	3718519	G	A	synonymous_variant	protein_coding	p.Ala44Ala

SO_3660	SO_3660	3814261	C	T	missense_variant	protein_coding	p.Pro467Leu
SO_3738	cysJ	3883943	G	A	missense_variant	protein_coding	p.Pro209Ser
SO_3984	SO_3984	4121397	A	G	missense_variant	protein_coding	p.Glu160Gly
SO_4047	SO_4047	4201277	G	A	synonymous_variant	protein_coding	p.Asp186Asp
SO_4087	SO_4087	4241326	T	C	synonymous_variant	protein_coding	p.Leu206Leu
SO_4091	tldD	4246867	T	C	missense_variant	protein_coding	p.Tyr120Cys
SO_4149	SO_4149	4312818	G	A	missense_variant	protein_coding	p.Ala1070Thr
SO_4149	SO_4149	4314603	G	A	missense_variant	protein_coding	p.Asp1665Asn
SO_4222	mraY	4396784	C	T	synonymous_variant	protein_coding	p.Lys358Lys
SO_4252	SO_4252	4428799	T	C	missense_variant	protein_coding	p.Val90Ala
SO_4312	cyaA	4490015	C	T	missense_variant	protein_coding	p.Ala140Thr
SO_4346	ilvM	4539373	A	G	missense_variant	protein_coding	p.Ser44Pro
SO_4369	SO_4369	4563811	G	A	missense_variant	protein_coding	p.Asp71Asn
SO_4377	SO_4377	4571503	T	C	missense_variant	protein_coding	p.Tyr248His
SO_4428	SO_4428	4619609	G	A	stop_gained	protein_coding	p.Gln72*

SO_4439	cooC	4628149	G	A	missense_variant	protein_coding	p.Glu341Lys
SO_4466	SO_4466	4659534	G	A	upstream_gene_variant		
SO_4618	SO_4618	4813216	C	T	missense_variant	protein_coding	p.Pro334Ser
SO_4713	menF	4917418	T	C	synonymous_variant	protein_coding	p.Asp287Asp
Group B							
SO_0024	trkH	29230	C	T	synonymous_variant	protein_coding	p.Val45Val
SO_0033	SO_0033	38321	A	G	missense_variant	protein_coding	p.Thr100Ala
SO_0455	SO_0455	481557	C	T	missense_variant	protein_coding	p.Gly261Asp
SO_0618	astA	645738	C	T	synonymous_variant	protein_coding	p.Asp314Asp
SO_0855	attH	876904	C	A	missense_variant	protein_coding	p.Gly99Cys
SO_1599	pfaC	1672246	G	A	missense_variant	protein_coding	p.Pro1321Leu
SO_4055	metL	4211107	T	C	missense_variant	protein_coding	p.Thr45Ala
SO_4652	sbp	4848425	T	C	missense_variant	protein_coding	p.Ile134Thr
Group C							
SO_0207	murI	214846	A	G	missense_variant	protein_coding	p.Ile12Val
SO_0220	rplK	230042	A	G	missense_variant	protein_coding	p.Lys81Arg

SO_0227	rpsG	241797	A	G	missense_variant	protein_coding	p.His153Arg
SO_0427	SO_0427	447995	T	C	missense_variant	protein_coding	p.Leu1056Pro
SO_0715	sorA	731939	C	T	missense_variant	protein_coding	p.Ala65Val
SO_0872	pcnB	902324	A	G	synonymous_variant	protein_coding	p.Asp207Asp
SO_0877	SO_0877	907389	C	T	synonymous_variant	protein_coding	p.Gly100Gly
SO_0915	SO_0915	942803	A	G	synonymous_variant	protein_coding	p.Phe468Phe
SO_0933	fba	965245	A	G	missense_variant	protein_coding	p.Ile332Val
SO_1114	dinB	1158220	T	C	synonymous_variant	protein_coding	p.Leu355Leu
SO_1669	tyrR	1755034	C	T	synonymous_variant	protein_coding	p.Gly25Gly
SO_2633	trmU	2769715	A	G	missense_variant	protein_coding	p.Asp332Gly
SO_2702	gpJ	2820047	G	A	missense_variant	protein_coding	p.Asp213Asn
SO_2767	asnB	2890314	T	C	synonymous_variant	protein_coding	p.Tyr331Tyr
SO_3455	relA	3600150	T	C	missense_variant	protein_coding	p.Glu53Gly
SO_3981	narQ	4119123	G	T	stop_gained	protein_coding	p.Glu423*
SO_4048	SO_4048	4202195	T	C	missense_variant	protein_coding	p.Ile93Val

SO_4053	SO_4053	4207416	G	C	missense_variant	protein_coding	p.Val552Leu
SO_4148	SO_4148	4308583	G	A	missense_variant	protein_coding	p.Ser104Asn
SO_4267	hsdR	4450880	C	T	upstream_gene_variant		
SO_4478	cpxA	4667042	A	G	missense_variant	protein_coding	p.Asp403Gly
SO_4702	gor	4904192	T	C	missense_variant	protein_coding	p.Tyr153His
SO_4723	mobB/moeA	4927078	T	C	synonymous_variant	protein_coding	p.Ile11Ile

Table S7. Nonsynonymous gene mutations of integral components of the membrane.

Locus tag	Gene	Gene product /Mutation type	Genome position	Base change	Amino acid change
Population D40Cr					
SO_0174	*gspL*	T2aSSsecretion system inner membrane platform protein (missense variant)	181023	T→C	Met(197) → Thr
SO_3313	*rodZ*	transmembrane cell shape protein(missense_variant)	3465177	T→C	Thr(302) → Ala
SO_0449	–	iron-regulated inner membrane protein(stop-gain)	476335	C→T	–
Population D80Cr					
SO_4377	–	MMPL family efflux pump permease component (missense_variant)	4572251	G→A	Gly(497) → Asp
SO_3492	*mexF*	HAE1 family efflux pump permease component (missense_variant)	3642994	A→G	Val(40) → Ala
SO_0174	*gspL*	T2aSSsecretion system inner membrane platform protein(missense_variant)	181023	T→C	Met(197) → Thr

SO_4657	–	diguanylate cyclase/phosphodiesterase with PAS and LOV sensory domains and Chase small molecule binding site (missense_variant)	4853822	A→G	Ile(736) → Thr
SO_0308	–	inner membrane protein of unknown function (missense_variant)	313497	A→G	Phe(238) → Leu
SO_4645	–	putative bifunctional autotransporter / extracellular effector(missense_variant)	4842096	T→G	Glu(40) → Ala
SO_4150	–	sulphate transporter(missense_variant)	4325377	T→C	Phe(157) → Ser
SO_3092	–	transmembrane protein in aerotolerance operon (missense_variant)	3217017	C→A	Ala(164) → Asp
Population D120Cr					
SO_0754	*atmA*	ABC-type Ni2+/Co2+ export system bifunctional ATPase and permease components (missense_variant)	769746	T→C	Thr(481) →Ala
SO_1034	*btuC*	ABC-type cobalamin uptake system permease component (missense_variant)	1073664	A→G	Ser(235)→ Gly
SO_0856	*attFG*	ABC-type export system permease component (missense_variant)	879614	C→T	Gly(92)→ Asp
SO_0822	*macC*	ABC-type macrolide export system secretin component(missense_variant)	838336	A→C	Gln(57)→ Pro

SO_4377	–	MMPL family efflux pump permease component (missense_variant)	4571503	T→C	Tyr(248)→ His
SO_3279	–	RND superfamily efflux pump permease component(missense_variant)	3419927	A→T	Leu(322)→ Phe
SO_0455	–	alpha-ketoglutarate uptake system bifunctional large and small subunit permease component (missense_variant)	481557	C→T	Gly(261)→ Asp
SO_0189	–	bifunctional autotransporter / adhesin cadherin family(missense_variant)	197007	C→T	Ala(1507) →Val
SO_0812	–	hypothetical protein(missense_variant)	824046	T→C	Leu(53)→ Pro
SO_4053	–	methyl-accepting chemotaxis protein (missense_variant)	4207416	G→C	Val(552) → Leu
SO_3981	*narQ*	nitrate/nitrite-responsive two component signal transduction system histidine kinase(stop-gain)	4119123	G→T	–
SO_4478	*cpxA*	periplasmic stress-responsive two component signal transduction system histidine kinase (missense_variant)	4667042	A→G	Asp(403) → Gly
SO_4148	–	type I protein secretion system MFP component HlyD family(missense_variant)	4308583	G→A	Ser(104) → Asn