

**Supplemental Table 1 Strains**

<b>Strain</b>	<b>Genotype</b>	<b>Reference</b>
<i>E. coli</i> Top10 DH5a	F <sup>-</sup> <i>mcrA</i> Δ( <i>mrr-hsdRMS-mcrBC</i> ) φ80 <i>lacZ</i> ΔM15 Δ <i>lacX74</i> <i>recA1</i> <i>araD139</i> Δ( <i>ara-leu</i> ) 7697 <i>galU</i> <i>galK</i> <i>rpsL</i> (Str <sup>R</sup> ) <i>endA1</i> <i>nupG</i> λ-	Invitrogen, CA
<i>E. coli</i> <i>inv110</i>	F' { <i>tra</i> Δ36 <i>proAB</i> <i>lacI<sub>q</sub></i> <i>lacZ</i> ΔM15} <i>rpsL</i> (Str <sup>R</sup> ) <i>thr</i> <i>leu</i> <i>endA</i> <i>thi-1</i> <i>lacY</i> <i>galK</i> <i>galT</i> <i>ara</i> <i>tonA</i> <i>tsx</i> <i>dam</i> <i>dcm</i> <i>supE44</i> Δ( <i>lac-proAB</i> ) Δ( <i>mcrC-mrr</i> )102::Tn10 (Tet <sup>R</sup> )	Invitrogen, CA
<i>H. volcanii</i> DS70		[5]
<i>H. volcanii</i> H26	DS70 Δ <i>pyrE2</i>	[1]
VDC2177	H26 Δ <i>HVO_2736</i>	[2]
VDC3226	H26 pJAM202c	This work
VDC2329	H26 Δ <i>HVO_1105</i>	This work
VDC2347	H26 Δ <i>HVO_0156</i>	This work
VDC2364	H26 Δ <i>HVO_2493</i>	This work
VDC2385	H26 Δ <i>HVO_0236</i>	This work
VDC2399	H26 Δ <i>HVO_B0354</i>	This work

VDC2414	H26 $\Delta$ HVO_0390	This work
VDC2452	H26 $\Delta$ HVO_2888	This work
VDC2457	H26 $\Delta$ HVO_0580	This work
VDC2459	H26 $\Delta$ HVO_1852	This work
VDC2469	H26 $\Delta$ HVO_1475	This work
VDC2476	H26 $\Delta$ HVO_2478	This work
VDC2506	H26 $\Delta$ HVO_0658	This work
VDC2514	H26 $\Delta$ HVO_2744	This work
VDC2517	H26 $\Delta$ HVO_0916	This work
VDC2527	H26 $\Delta$ HVO_1631	This work
VDC2545	H26 $\Delta$ HVO_2906	This work
VDC3241	H26 $\Delta$ HVO_2001	[2]
VDC3347	H26 $\Delta$ HVO_1717	This work
VDC3352	H26 $\Delta$ HVO_1716	This work
VDC3259	H26 $\Delta$ HVO_2001 pJAM202c	This work
VDC3265	H26 $\Delta$ HVO_2001 pGP109	This work
VDC3414	H26 $\Delta$ HVO_1105 $\Delta$ HVO_2008	This work
VDC5203	H26 $\Delta$ HVO_2008	This work
VDC2554	H26 $\Delta$ HVO_0253 pIKB437	This work
VDC2552	H26 $\Delta$ HVO_2747 pIKB434	This work
VDC2556	H26 $\Delta$ HVO_0339 pIKB434	This work

**Supplemental Table 2 Plasmids**

<b>Plasmid</b>	<b>Description</b>	<b>Reference</b>
pJAM202	<i>H. volcanii</i> shuttle vector	[3]
pTA131	Amp <sup>R</sup> ; ColE1	[1]
pBY158	pTA131 derivative containing the attCm <sup>R</sup> Ccd <sup>R</sup> cassette	[2]
pBY165.31	pTA131 $\Delta$ HVO_2001	[2]
pNAB135	pTA131 $\Delta$ HVO_2008	[4]
pGP068	pBY158 $\Delta$ HVO_2747	This work
pGP041	pCR8/GW 1000BpUpDn $\Delta$ HVO_0929	This work
pGP019	pCR8/GW 1000BpUPDn HVO_0929	This work
pIKB264	pTA131 $\Delta$ HVO_1383	This work
pIKB103	pTA131 $\Delta$ HVO_2736	[2]
pIKB154	pTA131 $\Delta$ HVO_0156	This work
pIKB156	pTA131 $\Delta$ HVO_0574	This work
pIKB162	pTA131 $\Delta$ HVO_1105	This work
pIKB172	pTA131 $\Delta$ HVO_1594	This work
pIKB180	pTA131 $\Delta$ HVO_1979	This work
pIKB184	pTA131 $\Delta$ HVO_1173	This work
pIKB199	pTA131 $\Delta$ HVO_1852	This work

pIKB203	pTA131 $\Delta$ HVO_2888	This work
pIKB207	pTA131 $\Delta$ HVO_1475	This work
pIKB211	pTA131 $\Delta$ HVO_0697	This work
pIKB215	pTA131 $\Delta$ HVO_0236	This work
pIKB219	pTA131 $\Delta$ HVO_2493	This work
pIKB229	pTA131 $\Delta$ HVO_0339	This work
pIKB240	pTA131 $\Delta$ HVO_0580	This work
pIKB251	pTA131 $\Delta$ HVO_2906	This work
pIKB262	pTA131 $\Delta$ HVO_B0354	This work
pIKB265	pTA131 $\Delta$ HVO_0253	This work
pIKB268	pTA131 $\Delta$ HVO_1717	This work
pIKB271	pTA131 $\Delta$ HVO_3412	This work
pIKB274	pTA131 $\Delta$ HVO_0390	This work
pIKB276	pTA131 $\Delta$ HVO_1716	This work
pIKB290	pTA131 $\Delta$ HVO_1173	This work
pIKB304	pTA131 $\Delta$ HVO_2478	This work
pIKB315	pTA131 $\Delta$ HVO_0658	This work
pIKB415	pTA131 $\Delta$ HVO_1631	This work
pIKB417	pTA131 $\Delta$ HVO_0916	This work
pIKB418	pTA131 $\Delta$ HVO_2744	This work
pIKB371	HVO_0339 inserted between the <i>NdeI</i> <i>BlnI</i> sites of pJAM202c	This work

pIKB437	<i>HVO_0253</i> inserted between the <i>NdeI</i> <i>BlpI</i> sites of pJAM202c	This work
pIKB367	<i>HVO_1979</i> inserted between the <i>NdeI</i> <i>BlpI</i> sites of pJAM202c	This work
pIKB434	<i>HVO_2747</i> inserted between the <i>NdeI</i> <i>BlpI</i> sites of pJAM202c	This work
pGP109	<i>HVO_2001</i> inserted between the <i>NdeI</i> and <i>BlpI</i> sites of pJAM202	This work

**Supplemental Table 3 Oligonucleotides**

Oligonucleotide	Sequence	Purpose
<b>Deletion constructs using In-fusion</b>		
HVO_1631_NF	cgggccccccctcgaggaggaagaccgtcgaaacc	Construction of <i>HVO_1631</i> deletion construct, pIKB415
HVO_1631_NR	gacgcgttcatatgcgttcacaagccgatgct	
HVO_1631_CF	gcatatgaacgcgtctcgacgatctgttcgagttc	
HVO_1631_CR	cgggctgcaggaattcgtaatcgcgcctgactg	
HVO_0916_NF	cgggccccccctcgagcatcgaatcactcggctaca	Construction of <i>HVO_0916</i>
HVO_0916_NR	gacgcgttcatatgcgtccaccacgtcgaagc	

HVO_0916_CF	gcatatgaacgcgtccctcccgccttcgac	deletion
HVO_0916_CR	cgggctgcaggaattcaggatgccgccttcgag	construct, pIKB417
HVO_2744_NF	cgggccccctcgagacggcgtcgaggaactc	Construction
HVO_2744_NR	gacgcgttcatatgcgaggtcgtcgttcaggagtc	of <i>HVO_2744</i>
HVO_2744_CF	gcatatgaacgcgtcggctgtagaccgaggtgtt	deletion
HVO_2744_CR	cgggctgcaggaattctctcccaccgttcgaaat	construct, pIKB418
HVO_2478_N_Fwd	cgggccccctcgaggaactgcgggcgaag	Construction
HVO_2478_N_Rev	gacgcgttcatatgccgacgaaagcgaggtgtc	of <i>HVO_2478</i>
HVO_2478_C_Fwd	gcatatgaacgcgtcgtcgtcaggctcgcaacg	deletion
HVO_2478_C_Rev	cgggctgcaggaattcgtccacatcactgcacct	construct, pIKB304
HVO_1716_N_Fwd	cgggccccctcgagcgcgacgactaccactg	Construction of
HVO_1716_N_Rev	gacgcgttcatatgcgacgtgggttcggtagtca	<i>HVO_1716</i>
HVO_1716_C_Fwd	gcatatgaacgcgtcgcgacccgattccgta	deletion
HVO_1716_C_Rev	cgggctgcaggaattcgttctcgaaggcgaag	construct, pIKB276
HVO_1717_N_Fwd	cgggccccctcgaggagcgacctagaacgacacc	Construction
HVO_1717_N_Rev	gacgcgttcatatgcgcgaacagctcgtttatcg	of <i>HVO_1717</i>
HVO_1717_C_Fwd	gcatatgaacgcgtccgactccacgtcgacctct	deletion
HVO_1717_C_Rev	cgggctgcaggaattctcgaaggcgtcgaagaact	construct, pIKB268

HVO_339_N_IfKO_Fwd	cgggccccccctcgagtcgaggctcacgtacgtcc	Construction of <i>HVO_339</i> deletion construct, pIKB229
HVO_339_N_IfKO_Rev	gacgcgttcatatgccgccagataggtcgtacaca	
HVO_339_C_IfKO_Fwd	gcatatgaacgcgtcatcgttaagccgctcgtt	
HVO_339_C_IfKO_Rev	cgggctgcaggaattcacggccatgctcgatatgt	
HVO_1475_N_IfKO_Fwd	cgggccccccctcgagagtcgacgacgaccaaac	Construction of <i>HVO_1475</i> deletion construct, pIKB207
HVO_1475_N_IfKO_Rev	gacgcgttcatatgcagttcactccacgacgag	
HVO_1475_C_IfKO_Fwd	gcatatgaacgcgtcggctactgttcgagcgagacct	
HVO_1475_C_IfKO_Rev	cgggctgcaggaattcgaaccaccccgtagatta	
HVO_0697_N_IfKO_Fwd	cgggccccccctcgagccctggaggctgactacg	Construction of <i>HVO_0697</i> deletion construct, pIKB211
HVO_0697_N_IfKO_Rev	gacgcgttcatatgctcaggagatgcaacagcaac	
HVO_0697_C_IfKO_Fwd	gcatatgaacgcgtccagccctcgatgaggac	
HVO_0697_C_IfKO_Rev	cgggctgcaggaattcgtcccctacgtcaacgtcac	
HVO_0253_N_IfKO_Fwd	cgggccccccctcgaggtaacgcggagtcttg	Construction of <i>HVO_0253</i> deletion construct, pIKB265
HVO_0253_N_IfKO_Rev	gacgcgttcatatgcgtctcggtcgggtagacg	
HVO_0253_C_IfKO_Fwd	gcatatgaacgcgtcgacatcgaggcgtggttaga	
HVO_0253_C_IfKO_Rev	cgggctgcaggaattcgtcgcgcacaaactcgtc	
HVO_0236_N_IfKO_Fwd	cgggccccccctcgaggtagccgtgtgcttacaga	Construction of <i>HVO_0236</i> deletion
HVO_0236_N_IfKO_Rev	gacgcgttcatatgccctgtgtggggttagaaga	
HVO_0236_C_IfKO_Fwd	gcatatgaacgcgtcgtcgatagatcgcgaggtgac	

HVO_0236_C_IfKO_Rev	cgggctgcaggaattcgcgagagcagttggagaaac	construct, pIKB215
HVO_2493_N_IfKO_Fwd	cgggccccctcgagcagagagcgacagttgacga	Construction of <i>HVO_2493</i> deletion
HVO_2493_N_IfKO_Rev	gacgcgttcatatgccgtctgtctggcgaact	
HVO_2493_C_IfKO_Fwd	gcatatgaacgcgtcgttctgcaggtgacgac	
HVO_2493_C_IfKO_Rev	cgggctgcaggaattcgcacccagcatggatattgt	construct, pIKB219
HVO_574_N_IfKO_Fwd	cgggccccctcgagcgcagggcagcagttc	Construction of <i>HVO_0574</i> deletion
HVO_574_N_IfKO_Rev	gacgcgttcatatgcgatttcggtcatctcgggta	
HVO_574_C_IfKO_Fwd	gcatatgaacgcgtccgaggatgcgctcgac	
HVO_574_C_IfKO_Rev	cgggctgcaggaattcgcgcagggcaacctc	construct, pIKB156
HVO_1979_N_IfKO_Fwd	cgggccccctcgagccgacgatggcgtgtt	Construction of <i>HVO_1979</i> deletion
HVO_1979_N_IfKO_Rev	gacgcgttcatatgcaggacgagccgttcgag	
HVO_1979_C_IfKO_Fwd	gcatatgaacgcgtcagacagggcgtcagacc	
HVO_1979_C_IfKO_Rev	cgggctgcaggaattccggagaacagaagcgagaag	construct, pIKB180
HVO_1105_N_IfKO_Fwd	cgggccccctcgagggccatgccgtagagacc	Construction of <i>HVO_1105</i> deletion
HVO_1105_N_IfKO_Rev	gacgcgttcatatgcccggaggtcgtccaaatc	
HVO_1105_C_IfKO_Fwd	gcatatgaacgcgtccgaggacgaagagtgag	
HVO_1105_C_IfKO_Rev	cgggctgcaggaattcagagaacgctgggcgaag	construct, pIKB162
HVO_1383_N_IfKO_Fwd	cgggccccctcgagagccgacgccgacac	Construction

HVO_1383_N_IfKO_Rev	gacgcgttcatatgcccgtggacgaggagtatcac	of <i>HVO_1383</i>
HVO_1383_C_IfKO_Fwd	gcatatgaacgcgtccaccggctacctcgtgtt	deletion
HVO_1383_C_IfKO_Rev	cgggctgcaggaattccgtggcgtttctgttc	construct, pIKB
HVO_0658_N_IfKO_Fwd	cgggccccctcgagcgtcgtggccatcttc	Construction
HVO_0658_N_IfKO_Rev	gacgcgttcatatgcgacgacctcgtcttcgactt	of <i>HVO_0658</i>
HVO_0658_C_IfKO_Fwd	gcatatgaacgcgtccgcacgcggaagtct	deletion
HVO_0658_C_IfKO_Rev	cgggctgcaggaattccacgagcgcgacgac	construct, pIKB315
HVO_1173_N_IfKO_Fwd	cgggccccctcgagagcttgaggagccgtagat	Construction
HVO_1173_N_IfKO_Rev	gacgcgttcatatgcgacgtgcgtcgtcatcc	of <i>HVO_1173</i>
HVO_1173_C_IfKO_Fwd	gcatatgaacgcgtcccgaaaaccgggtggt	deletion
HVO_1173_C_IfKO_Rev	cgggctgcaggaattcgaggtcgtcgttgcctc	construct, pIKB265
HVO_1852_N_IfKO_Fwd	cgggccccctcgaggcgcgtcgtgatgtg	Construction
HVO_1852_N_IfKO_Rev	gacgcgttcatatgcatccgcgaggagtcg	of <i>HVO_1852</i>
HVO_1852_C_IfKO_Fwd	gcatatgaacgcgtccggctgcgtggaac	deletion
HVO_1852_C_IfKO_Rev	cgggctgcaggaattcgttccgccctcgtta	construct, pIKB199
HVO_2906_N_IfKO_Fwd	cgggccccctcgagcgcacaccgtactttctcca	Construction
HVO_2906_N_IfKO_Rev	gacgcgttcatatgcgctgacgggcgtgttc	of <i>HVO_2906</i>
HVO_2906_C_IfKO_Fwd	gcatatgaacgcgtcccggtgttgccgtatc	deletion
HVO_2906_C_IfKO_Rev	cgggctgcaggaattccgagatattcaccgacgag	construct,

		pIKB251
HVO_0156_N_IfKO_Fwd	cgggccccccctcgagagcgacatcgaactctcg	Construction of <i>HVO_0156</i> deletion construct, pIKB154
HVO_0156_N_IfKO_Rev	gacgcgttcatatgcgtgcggtacgtgcatctg	
HVO_0156_C_IfKO_Fwd	gcatatgaacgcgtccccgaccagttcgacgac	
HVO_0156_C_IfKO_Rev	cgggctgcaggaattccgagagtggaactcgagtc	
HVO_1594_N_IfKO_Fwd	cgggccccccctcgaggatgcccagaggaacac	Construction of <i>HVO_1594</i> deletion construct, pIKB172
HVO_1594_N_IfKO_Rev	gacgcgttcatatgcaaaaaggcctcctcgtcgt	
HVO_1594_C_IfKO_Fwd	gcatatgaacgcgtccgtctaccgcaccacaac	
HVO_1594_C_IfKO_Rev	cgggctgcaggaattctattccggttgacgaggtc	
HVO_2747_N_IfKO_Fwd	cgggccccccctcgagccgaactcctttggaacat	Construction of <i>HVO_2747</i> deletion construct, pGP035
HVO_2747_N_IfKO_Rev	gacgcgttcatatgcgaaggaggtcatcgtcgag	
HVO_2747_C_IfKO_Fwd	gcatatgaacgcgtcgtggcggagtacgtcgag	
HVO_2747_C_IfKO_Rev	cgggctgcaggaattccgcttaacggtctcacc	
ORF02092_N_IfKO_Fwd	cgggccccccctcgagcgacggcgagcagttc	Construction of <i>HVO_2888</i> deletion construct, pIKB207
ORF02092_N_IfKO_Rev	gacgcgttcatatgcgatttcggtcatctcgggta	
ORF02092_C_IfKO_Fwd	gcatatgaacgcgtccgaggatgcgctcgac	
ORF02092_C_IfKO_Rev	cgggctgcaggaattcgcgcaggegaacctc	
HVO_580_N_IfKO_Fwd	cgggccccccctcgagcgccgttccggagctt	Construction of <i>HVO_0580</i>
HVO_580_N_IfKO_Rev	gacgcgttcatatgcgcacttgcgactccatt	

HVO_580_C_IfKO_Fwd	gcatatgaacgcgctcggaagtgccgactcatcg	deletion
HVO_580_C_IfKO_Rev	cgggctgcaggaattcgtggcctcgtccacatcac	construct, pIKB240
HVO_B0357_N_Fwd	cgggccccctcgagagcgcgaggtcgtactcc	Construction
HVO_B0357_N_Rev	gacgcgttcatatgcccgcgaggaacactacgg	of
HVO_B0357_C_Fwd	gcatatgaacgcgctccgtgatgactgagggtga	<i>HVO_B0357</i>
HVO_B0357_C_Rev	cgggctgcaggaattccgacgactggtacgagaaca	deletion construct, pIKB262
HVO_0339_N_Fwd	cgggccccctcgagtctatctccaccggtccttg	Construction
HVO_0339_N_Rev	gacgcgttcatatgvcgaggacttgacctcatac	of <i>HVO_0390</i>
HVO_0339_C_For	gcatatgaacgcgctccgacgacctcttcgagttct	deletion
HVO_0339_C_Rev	cgggctgcaggaattcgtgacgaacgtcggact	construct, pIKB274
<b>Deletion constructs, synthesised using methods other than In-Fusion</b>		
tgtA2HvUPF	tctagaggtgattaccacgcgcaag	Construction
tgtA2HvUPR	ggatccgcgtgaacctcgaagtagt	of <i>HVO_2008</i>
tgtA2HvDSol1	aatggatcctcgaagggttaattcgg	deletion
tgtA2HvDSol2	aatgcggccgcaagcttcggcagtcgcgtgttgatg	plasmid, described in [4]
UpDn_HVOB2736_fwd	ctg gag gat gtc gcc ctc gc	Construction

UpDn_HVO2736_rev	gac ggt cag gtg cgc ccc	of <i>HVO_2736</i> deletion plasmid, described in [2]
RevPCR_HVO2736_fwd	gacgcggccccgcaggaa	
RevPCR_HVO2736_rev	ttcggatcaggatggatac	
<b>Gene deletion verification</b>		
HVO_2478_Int_Fwd	gaggtactcggtcgctgtct	Internal checks for <i>HVO_2478</i>
HVO_2478_Int_Rev	ggattccctcgacgagttc	
HVO_2478_Ext_Fwd	acgtcgtacagcgtgcatc	External checks for <i>HVO_2478</i>
HVO_2478_Ext_Rev	gacgtggacaccgtcctct	
HVO_1105_Int_F	ccgacgcagacatcgaact	Internal checks for <i>HVO_1105</i>
HVO_1105_Int_R	ccgtctgaaagtcgagcatc	
HVO_1105_fwd	gcactgttgaacgacctct	External checks for <i>HVO_1105</i>
HVO_1105_rev	ctgtcggagcgaaaagagac	
HVO_B0357_Int_F	aggtcgtacgtctcgttcgt	Internal checks for <i>HVO_B0357</i>
HVO_B0357_Int_R	ggtcgggctgtacttctca	
HVO_B0357_Ext_F	cggacagtcgtatgtgcaac	External checks for
HVO_B0357_Ext_R	cgacgactggtacgagaaca	

		<i>HVO_B0357</i>
HVO_1594_int_Fwd	gcatcctggaactcgaagac	Internal
HVO_1594_int_Rev	aggtgcaggtggagtagacg	checks for <i>HVO_1594</i>
HVO_1594_ext_Fwd	cggcttaaactgtagactgc	External
HVO_1594_ext_Rev	ggactcagttcgatgacgttc	checks for <i>HVO_1594</i>
HVO_0253_Int_F	aacgcgtcttcgacatcaa	Internal
HVO_0253_Int_R	gtcgtcgtgctcgtctaacc	checks for <i>HVO_0253</i>
HVO_0253_Ext_F	cgggaccggaattaagagtt	External
HVO_0253_Ext_R	tgccatgtcagtaagccaag	checks for <i>HVO_0253</i>
HVO_2493_Int_Fwd	cgcttttgaggctgtggag	Internal
HVO_2493_Int_Rev	cgaaggctcgaagagtagc	checks for <i>HVO_2493</i>
HVO_2493_Ext_Fwd	gttcctcggtactccgtct	External
HVO_2493_Ext_Rev	gcctctgacagcagcaaata	checks for <i>HVO_2493</i>
HVO_1173_int_Fwd	gggtcatcctaccggacaac	Internal
HVO_1173_int_Rev	gccgggacgactttcttg	checks for <i>HVO_1173</i>
HVO_1173_Ext_fwd	gcgataccgacgaggagtc	External

HVO_1173_Ext_rev	ggtcctcgtagcagtccatc	checks for <i>HVO_1173</i>
HVO_1852_Int_F	cgaactcgacttccgggta	Internal
HVO_1852_Int_R	gtgtcccgactggtgtacg	checks for <i>HVO_1852</i>
HVO_1852_Ext_fwd	gagtacctcggcctcctctc	External
HVO_1852_Ext_rev	ctccaactcaggagcgaaag	checks for <i>HVO_1852</i>
HVO_2888_Int_F	cggaagtctccagacacat	Internal
HVO_2888_Int_R	tcgaacagcttcgacacatc	checks for <i>HVO_2888</i>
HVO_2888_Ext_fwd	gaagtccaagtgggcgacta	External
HVO_2888_Ext_rev	ctcggctacgagacgaagag	checks for <i>HVO_2888</i>
HVO_0580_Int_Fwd	ctctaccgagagttcacc	Internal
HVO_0580_Int_Rev	accggaacttctccagtt	checks for <i>HVO_0580</i>
HVO_0580_Fwd	ttcgcgatatgctcaatgaa	External
HVO_0580_Rev	cgcggtagagttcggagtag	checks for <i>HVO_0580</i>
HVO_0339_Int_F	ctctaccgagagttcacc	Internal
HVO_0339_Int_R	accggaacttctccagtt	checks for <i>HVO_0339</i>

HVO_0339_Ext_F	gttcatgccgtttctgact	External checks for <i>HVO_0339</i>
HVO_0339_Ext_R	cgacagtcacgtgaaatcg	
HVO_0697_Int_F	gttgctgttgcatctcctga	Internal checks for <i>HVO_0697</i>
HVO_0697_Int_R	ctaccacctcaccgacacct	
HVO_0697_Ext_F	cccgacgtagtgtccatct	External checks for <i>HVO_0697</i>
HVO_0697_Ext_R	gacaagccgacgaagaagac	
HvO_1383_Int_F	cggacctctcaaccacttc	Internal checks for <i>HVO_1383</i>
HvO_1383_Int_R	tcgttgaagtcacatctgctg	
HvO_1383_Ext_fwd	gctagcacggctcctacaac	External checks for <i>HVO_1383</i>
HvO_1383_Ext_rev	cggctgtgaactcctcttttc	
HVO_0156_Int_Fwd	ggtacttgacgaagccgaag	Internal checks for <i>HVO_0156</i>
HVO_0156_Int_Rev	acgctgtccaaggagttcag	
HVO_0156_Ext_Fwd	gatgcagcagatgcacgta	External checks for <i>HVO_0156</i>
HVO_0156_Ext_Rev	gacgtgagaggactcacc	
HVO_0658_Int_fwd	gtggagaccgactgtgtgg	Internal checks for
HVO_0658_Int_rev	cgaactcgactcgctgaac	

		<i>HVO_0658</i>
HVO_0658_Ext_fwd	gttcgggtggatacaggaga	External
HVO_0658_Ext_rev	agaactcgtggaggattgc	checks for <i>HVO_0658</i>
HVO_0236_Int_Fwd	caacgccctcatgtacgac	Internal
HVO_0236_Int_Rev	ccgaagtctctgtcacctc	checks for <i>HVO_0236</i>
HVO_0236_Ext_fwd	cggaccggaacatctcag	External
HVO_0236_Ext_rev	gtacgagcataccgttcgt	checks for <i>HVO_0236</i>
HVO_2906_Int_FWD	gagcaactcgtcgaaaaagc	Internal
HVO_2906_Int_Rev	acttcggcatctacgacctc	checks for <i>HVO_2906</i>
HVO_2906_Ext_fwd	agttcttgcggttgatggag	External
HVO_2906_Ext_rev	gacaccgagtcttcgacctc	checks for <i>HVO_2906</i>
HVO_1594_Int_Fwd	gcatcctggaactcgaagac	Internal
HVO_1594_Int_Rev	aggtgcaggtggagtagacg	checks for <i>HVO_1594</i>
HVO_1594_Ext_Fwd	ttcagcgaaccagacgctac	External
HVO_1594_Ext_Rev	cggcttttgattgggactg	checks for <i>HVO_1594</i>
HVO_1475_int_Fwd	aactccttgctcccttcgtt	Internal

HVO_1475_int_Rev	gtctcgctcgaacagtaccc	checks for <i>HVO_1475</i>
HVO_1475_Ext_fwd	gacgtgacagtcgaggtggt	External
HVO_1475_Ext_rev	agtacgcgtggagccactac	checks for <i>HVO_1475</i>
HVO_0574_Int_Fwd	agttcccgctggagacagt	Internal
HVO_0574_Int_Rev	cgaagtcgagtcgttcgtc	checks for <i>HVO_0574</i>
HVO_0574_Ext_fwd	agtccgagcctcgaagagtt	External
HVO_0574_Ext_rev	aggacagtcgcaagtccagt	checks for <i>HVO_0574</i>
HVO_1631_Int_Fwd	aggacctgtccctcgtaggt	Internal
HVO_1631_Int_Rev	aacagatcgtcgaagccatc	checks for <i>HVO_1631</i>
HVO_1631_Ext_Fwd	gctacctgtgccctgaatgt	External
HVO_1631_Ext_Rev	gggaactcctgtgcgataga	checks for <i>HVO_1631</i>
HVO_0916_Int_Fwd	ctcccagtcgaccttgatgt	Internal
HVO_0916_Int_Rev	tcgcggagttctacacgag	checks for <i>HVO_0916</i>
HVO_0916_Ext_Fwd	cgtccacaagagctacatcg	External
HVO_0916_Ext_Rev	gggtaatctcacgcaatggt	checks for <i>HVO_0916</i>

HVO_2744_Int_Fwd	tcgtagccggtgaagctact	Internal checks for <i>HVO_2744</i>
HVO_2744_Int_Rev	gggatgaacacctcggctcta	
HVO_2744_Ext_Fwd	gtcttcgaccagcgtgttct	External checks for <i>HVO_2744</i>
HVO_2744_Ext_Rev	gtgcgcccgtggatagac	
HvO_0253_Ext_F	cgggaccggaattaagagtt	External checks for <i>HVO_0253</i>
HvO_0253_Ext_R	tgccatgtcagtaagccaag	
HvO_0253_Int_F	aacgcgtcttcgacatcaa	Internal checks for <i>HVO_0253</i>
HvO_0253_Int_R	gtcgtcgtgctcgtctaacc	
HVO_1979_Ext_Fwd	cgacgattctcgaagctgat	External checks for <i>HVO_1979</i>
HVO_1979_Ext_Rev	gtcgagcaggacgaagtagg	
HVO_1979_Int_Fwd	gggtgtactgctcgatggtc	Internal checks for <i>HVO_1979</i>
HVO_1979_Int_Rev	gtgcagttcctcctcgacat	
HVO_2747_Int_Fwd	aaaagtcccgttccgctact	Internal checks for <i>HVO_2747</i>
HVO_2747_Int_Rev	acgaaatcctcaacgtcgtc	
HVO_2747_Ext_Fwd	gcgaggttcgattcgaggtc	External checks for
HVO_2747_Ext_Rev	tcgaggcgaggcgaagagc	

		<i>HVO_2747</i>
HVO_1717_Ext_Fwd	tcgcgcaccgcaatgagctt	External checks for
HVO_1717_Ext_Rev	aggttctggaacgcctgcaa	<i>HVO_1717</i>
HVO_1717_Int_Fwd	acgagctgttcgcgtcgctt	Internal checks for
HVO_1717_Int_Rev	acacgtcgtgtcgcggatt	<i>HVO_1717</i>
HVO_1716_Ext_Fwd	gccgtcgtgctcgaagagaa	External checks for
HVO_1716_Ext_Rev	ggcgactctgctcacctact	<i>HVO_1716</i>
HVO_1717_Int_Fwd	acgaactctacgcgtccac	Internal checks for
HVO_1717_Int_Rev	ggcgactctgctcacctact	<i>HVO_1716</i>
HVO_0929_Int_Fwd	tgctcgtcggcgactcgacc	External checks for
HVO_0929_Int_Rev	tcgcgaacgtcgcctgaat	<i>HVO_0929</i>
HVO_0929_Ext_Fwd	gaataaaaccgaaccgggtcgc	External checks for
HVO_0929_Ext_Rev	ggcatcgcgaaggaaatcaac	<i>HVO_0929</i>
<b>pJAM202c cloning</b>		
HVO_0253_NdeI_Fwd	atgccatatgatgagcgcacgactaca	Cloning of
HVO_0253_BlnI_Rev	atgcgctgagttacgcgtcgtcgtgct	<i>HVO_0253</i> into pJAM202

Hvo_2747_ndeI_fwd	atgccatatgatgacacgtacggagagcgg	Cloning
Hvo_2747_blpI_rev	atgcgctgagcctgtccgtcgcgtccgacga	<i>HVO_2747</i> into pJAM
HVO_0339_NdeI_Fwd	atgccatatgatgaccgtcatcggcctcga	Cloning of
HVO_0339_BlpI_Rev	atgcgctgagtcagcgtccgggtgcgt	<i>HVO_0339</i> into pJAM202
Hvo697_fwd	atgccatatgatggacgacatcgaatcga	Cloning of
Hvo697_rev	atgcgctgagtcagccgatgtaccggaggt	<i>HVO_0697</i> into pJAM202
HVO_1979_NdeI_Fwd	atgccatatgatgccatatgatgagcatccttgagg	Cloning of
HVO_1979_BlpI_Rev	atgcgctgagatgcgctcagctcagtcctgaagaa	<i>HVO_1979</i> into pJAM202
HVO_2001_Fwd	gctcagctacgtcgggtgaacctaaaga	Cloning of
HVO_2001_Rev	catatgcgcgaccacttcgaactc	<i>HVO_2001</i> into pJAM202

**Supplemental Table 4 Salt and temperature tolerance of tested deletion mutants**

**26°C**

<b>Strain</b>	<b>12%</b>	<b>14%</b>	<b>16%</b>	<b>18%</b>	<b>23%</b>	<b>25%</b>
<b>H26</b>	+	+	+	+	-	-
<i>HVO_0916</i>	-	-	-	-	-	-
<i>HVO_1631</i>	-	-	-	-	-	-
<i>HVO_2477</i>	+	++	++	++	-	-
<i>HVO_2493</i>	+	+	+	+	-	-
<i>HVO_1105</i>	+	+	+	+	-	-
<i>HVO_1105</i>	+	+	+	+	-	-
<i>HVO_2008</i>						
<i>HVO_2001</i>	-	-	-	-	-	-
<i>HVO_2008</i>	+	+	+	+	-	-
<i>HVO_1717</i>	+	+	+	+	-	-
<i>HVO_1716</i>	+	+	+	+	-	-
<i>HVO_0253</i>	+	+	+	+	-	-
<i>HVO_1803</i>	+	+	+	+	-	-
<i>HVO_B0354</i>	+	+	+	+	-	-
<i>HVO_1594</i>	+	+	+	+	-	-
<i>HVO_0390</i>	+	+	+	+	-	-
<i>HVO_2888</i>	+	+	+	+	-	-
<i>HVO_1378</i>	+	+	+	+	-	-
<i>HVO_1852</i>	+	+	+	+	-	-

<i>HVO_0658</i>	+	+	+	+	-	-
<i>HVO_2478</i>	+	+	+	+	-	-
<i>HVO_2736</i>	+	+	+	+	-	-
<i>HVO_1475</i>	+	+	+	+	-	-
<i>HVO_0236</i>	+	+	+	+	-	-

30°C

<b>Strain</b>	<b>12%</b>	<b>14%</b>	<b>16%</b>	<b>18%</b>	<b>23%</b>	<b>25%</b>
<b>H26</b>	++	++	++	++	-	-
<i>HVO_0916</i>	-	-	-	-	-	-
<i>HVO_1631</i>	++	++	++	++	-	-
<i>HVO_2477</i>	++	++	++	++	-	-
<i>HVO_2493</i>	++	++	++	++	-	-
<i>HVO_1105</i>	++	++	++	++	-	-
<i>HVO_1105</i>	++	++	++	+	-	-
<i>HVO_2008</i>						
<i>HVO_2001</i>	++	++	++	++	-	-
<i>HVO_2008</i>	++	++	++	++	-	-
<i>HVO_1717</i>	++	++	++	++	-	-
<i>HVO_1716</i>	++	++	++	++	-	-
<i>HVO_0253</i>	++	++	++	++	-	-
<i>HVO_1803</i>	++	++	++	++	-	-
<i>HVO_B0354</i>	++	++	++	++	-	-

<i>HVO_1594</i>	++	++	++	++	-	-
<i>HVO_0390</i>	++	++	++	++	-	-
<i>HVO_2888</i>	++	++	++	++	-	-
<i>HVO_1378</i>	++	++	++	++	-	-
<i>HVO_1852</i>	++	++	++	++	-	-
<i>HVO_0658</i>	++	++	++	++	-	-
<i>HVO_2478</i>	++	++	++	++	-	-
<i>HVO_2736</i>	++	++	++	++	-	-
<i>HVO_1475</i>	++	++	++	++	-	-
<i>HVO_0236</i>	++	++	++	++	-	-

37°C

Strain	12%	14%	16%	18%	23%	25%
<b>H26</b>	++	++	++	++	-	-
<i>HVO_0916</i>	-	-	-	-	-	-
<i>HVO_1631</i>	++	++	++	++	-	-
<i>HVO_2477</i>	++	++	++	++	-	-
<i>HVO_2493</i>	++	++	++	++	-	-
<i>HVO_1105</i>	++	++	++	++	-	-
<i>HVO_1105</i> <i>HVO_2008</i>	++	++	++	+	-	-
<i>HVO_2001</i>	++	++	++	++	-	-
<i>HVO_2008</i>	++	++	++	++	-	-

<i>HVO_1717</i>	++	++	++	++	-	-
<i>HVO_1716</i>	++	++	++	++	-	-
<i>HVO_0253</i>	++	++	++	++	-	-
<i>HVO_1803</i>	++	++	++	++	-	-
<i>HVO_B0354</i>	++	++	++	++	-	-
<i>HVO_1594</i>	++	++	++	++	-	-
<i>HVO_0390</i>	++	++	++	++	-	-
<i>HVO_2888</i>	++	++	++	++	-	-
<i>HVO_1378</i>	++	++	++	++	-	-
<i>HVO_1852</i>	++	++	++	++	-	-
<i>HVO_0658</i>	++	++	++	++	-	-
<i>HVO_2478</i>	++	++	++	++	-	-
<i>HVO_2736</i>	++	++	++	++	-	-
<i>HVO_1475</i>	++	++	++	++	-	-
<i>HVO_0236</i>	++	++	++	++	-	-

44°C

<b>Strain</b>	<b>12%</b>	<b>14%</b>	<b>16%</b>	<b>18%</b>	<b>23%</b>	<b>25%</b>
<b>H26</b>	++	++	++	++	++	+
<i>HVO_0916</i>	+	+	++	++	+	-
<i>HVO_1631</i>	++	++	++	++	++	-
<i>HVO_2477</i>	++	++	++	++	++	+
<i>HVO_2493</i>	++	++	++	++	++	+

<i>HVO_1105</i>	++	++	++	++	++	+
<i>HVO_1105</i> <i>HVO_2008</i>	++	++	++	++	++	+
<i>HVO_2001</i>	++	++	++	++	++	+
<i>HVO_2008</i>	++	++	++	++	++	+
<i>HVO_1717</i>	++	++	++	++	++	+
<i>HVO_1716</i>	++	++	++	++	++	+
<i>HVO_0253</i>	++	++	++	++	++	+
<i>HVO_1803</i>	++	++	++	++	++	+
<i>HVO_B0354</i>	++	++	++	++	++	+
<i>HVO_1594</i>	++	++	++	++	++	+
<i>HVO_0390</i>	++	++	++	++	++	+
<i>HVO_2888</i>	++	++	++	++	++	+
<i>HVO_1378</i>	++	++	++	++	++	+
<i>HVO_1852</i>	++	++	++	++	++	+
<i>HVO_0658</i>	++	++	++	++	++	+
<i>HVO_2478</i>	++	++	++	++	++	+
<i>HVO_2736</i>	++	++	++	++	++	+
<i>HVO_1475</i>	++	++	++	++	++	+
<i>HVO_0236</i>	++	++	++	++	++	+

50°C

<b>Strain</b>	<b>12%</b>	<b>14%</b>	<b>16%</b>	<b>18%</b>	<b>23%</b>	<b>25%</b>
---------------	------------	------------	------------	------------	------------	------------

<b>H26</b>	-	-	-	++	++	++
<i>HVO_0916</i>	-	-	-	+	+	+
<i>HVO_1631</i>	-	-	-	++	++	++
<i>HVO_2477</i>	-	-	-	++	++	++
<i>HVO_2493</i>	-	-	-	++	++	++
<i>HVO_1105</i>	-	-	-	++	++	++
<i>HVO_1105</i>	-	-	-	++	++	++
<i>HVO_2008</i>						
<i>HVO_2001</i>	-	-	-	++	++	++
<i>HVO_2008</i>	-	-	-	++	++	++
<i>HVO_1717</i>	-	-	-	++	++	++
<i>HVO_1716</i>	-	-	-	++	++	++
<i>HVO_0253</i>	-	-	-	++	++	++
<i>HVO_1803</i>	-	-	-	++	++	++
<i>HVO_B0354</i>	-	-	-	++	++	++
<i>HVO_1594</i>	-	-	-	++	++	++
<i>HVO_0390</i>	-	-	-	++	++	++
<i>HVO_2888</i>	-	-	-	++	++	++
<i>HVO_1378</i>	-	-	-	++	++	++
<i>HVO_1852</i>	-	-	-	++	++	++
<i>HVO_0658</i>	-	-	-	++	++	++
<i>HVO_2478</i>	-	-	-	++	++	++
<i>HVO_2736</i>	-	-	-	++	++	++

<b>HVO_1475</b>	-	-	-	++	++	++
<b>HVO_0236</b>	-	-	-	++	++	++

## References

1. **Allers, T., H. Ngo, M. Mevarech, and R. Lloyd.** 2004. Development of additional selectable markers for the halophilic archaeon *Haloferax volcanii* based on the *leuB* and *trpA* genes. *Appl Environ Microbiol* **70**:943-53.
2. **El Yacoubi, B., G. Phillips, I. Blaby, C. Haas, Y. Cruz, J. Greenberg, and V. de Crécy-Lagard.** 2009. A Gateway platform for functional genomics in *Haloferax volcanii*: deletion of three tRNA modification genes. *Archaea* **2**:211-9.
3. **Kaczowka, S., and J. Maupin-Furlow.** 2003. Subunit topology of two 20S proteasomes from *Haloferax volcanii*. *J Bacteriol* **185**:165-74.
4. **Phillips, G., V. Chikwana, A. Maxwell, B. El-Yacoubi, M. Swairjo, D. Iwata-Reuyl, and V. de Crécy-Lagard.** 2010. Discovery and characterization of an amidinotransferase involved in the modification of archaeal tRNA. *J Biol Chem* **285**:12706-13.
5. **Wendoloski, D., C. Ferrer, and M. Dyll-Smith.** 2001. A new simvastatin (mevinolin)-resistance marker from *Haloarcula hispanica* and a new *Haloferax volcanii* strain cured of plasmid pHV2. *Microbiology* **147**:959-64.