

**Supplementary Information:**

**Membrane vesicle-mediated release of bacterial RNA**

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## **Legends to Supplementary figures**

### **Supplementary Data Fig. S1.**

A) Analyses of RNA extracted from a log-phase ( $OD = 0.7$ ) culture of *Vibrio cholerae* O1 El Tor strain A1552. Left panel: RNA extracted from whole cells (lane 1) and vesicles (lane 2) using the Total RNA Norgen kit. 1.5  $\mu$ g RNA were run on a 12 % polyacrylamide denaturing gel stained in GelRed (light red). Right panel: RNA was transferred to a membrane for Northern blot analysis and probed with a probe for gene *vc2478,5* (lane 3; whole cells and lane 4: vesicles)

B) Western blot analysis of using antibodies recognizing RpoS to check for possible lysis of bacteria. Lane 1: whole cell extract from overnight culture; lane 2: supernatant from overnight culture after filtration through 0.22  $\mu$ m steripup filters; lane 3: supernatant from overnight culture after ultracentrifugation; lane 4: vesicles from overnight culture; lane 5: whole cell extract from a culture at  $OD_{600} = 3$  and lane 6: whole cell extract from A1552 RpoS mutant.

C) Fluorescence microscopy analysis of bacteria after live/dead staining. Staining was performed using the LIVE/DEAD® BacLight™ Bacterial Viability Kit L13152 with bacterial samples from *V. cholerae* O1 El Tor strain A1552 cultures as follows: a) sample from  $OD_{600} = 0.7$  (log-phase); b) sample from  $OD_{600} = 4.6$  (overnight culture); c) sample from  $OD_{600} = 4.6$  (overnight culture) after treatment with isopropanol to obtain a population of only dead bacteria for comparison.

**Supplementary Data Fig. S2.** Density gradient centrifugation purification of vesicles from an overnight culture of the *V. cholerae* O1 El Tor strain A1552. **A.** Left panel: Photo of the

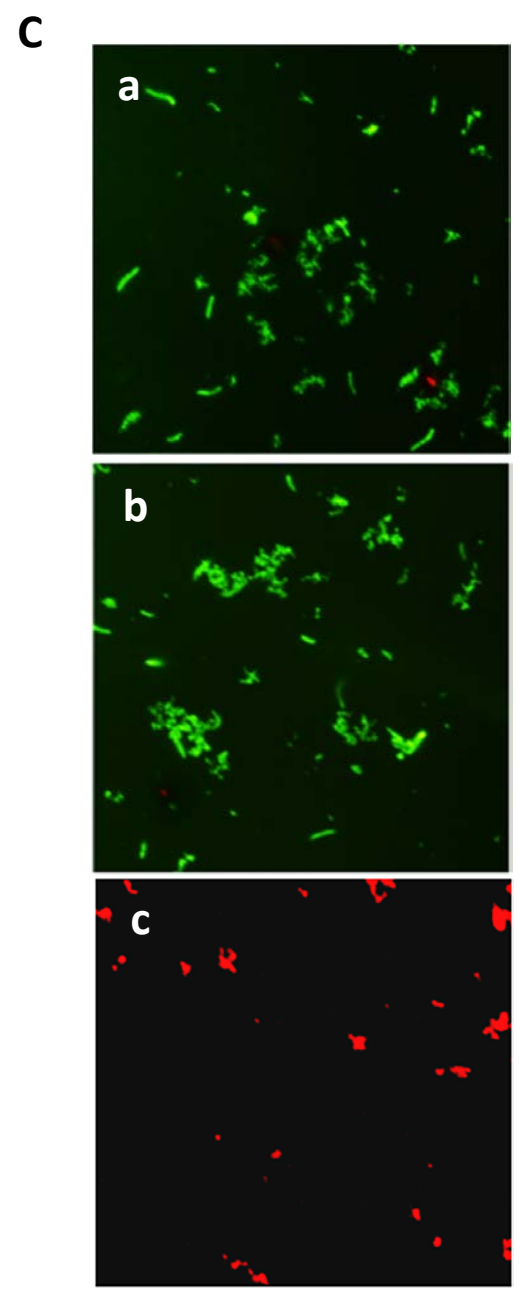
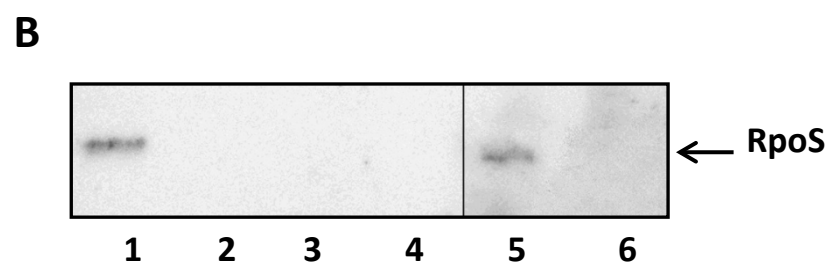
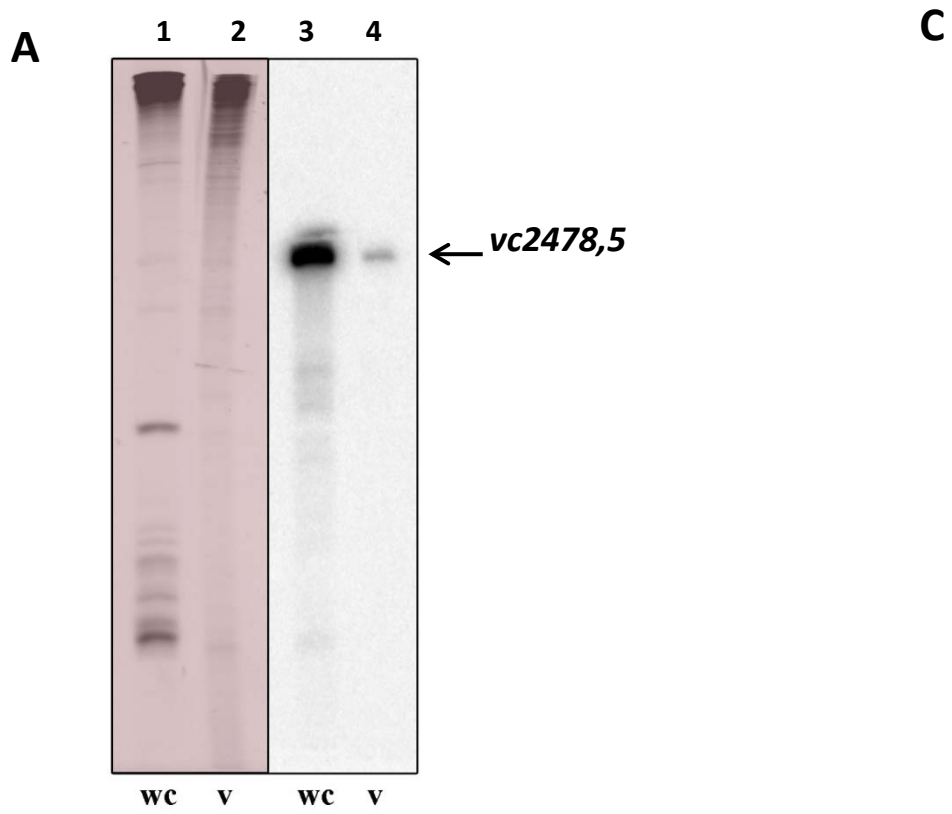
density gradient tube after ultracentrifugation of a vesicle sample. The five visible rings (fractions) are numbered 1 to 5 in increasing density order. Right panel: Western blot analysis of samples from each numbered fraction using antibodies recognizing OmpU (VC 0633, Outer membrane protein U, 37kDa).

**B.** Analyses of RNA extracted from each of the five density gradient fractions in A. RNA extracts obtained using the Total RNA Norgen kit were separated on a 12 % polyacrylamide denaturing gel. The gel was stained and subsequently blotted for transfer to a membrane. Left panel: Gel stained with GelRed. Right panel: Northern blot membrane probed with probe *vc2478,5*. Lanes: *wc*, whole cell RNA extract; 1 - 5: samples originating from each of the numbered density gradient fractions described in A.

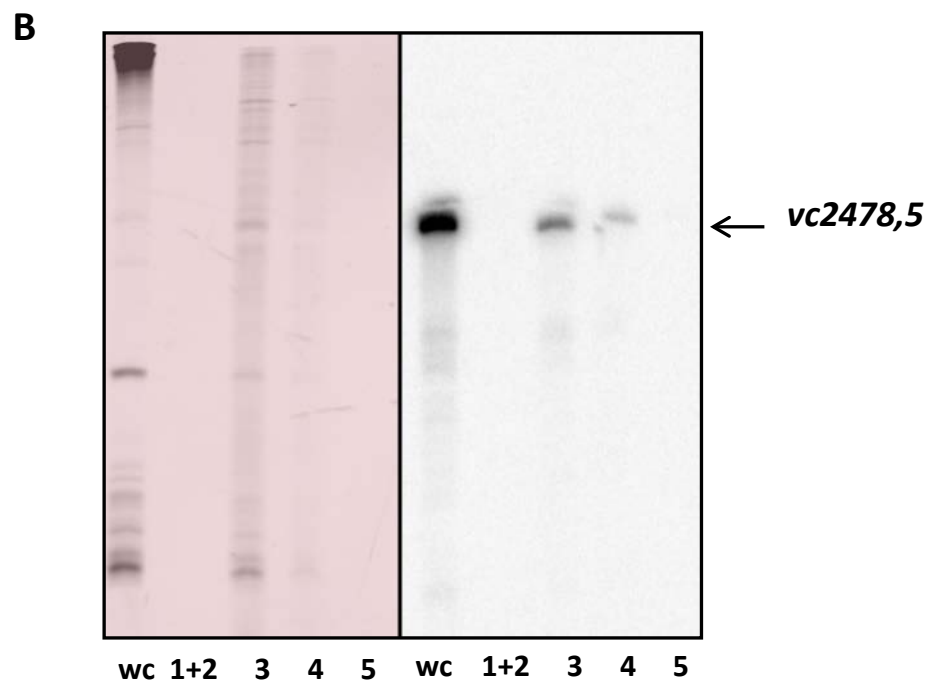
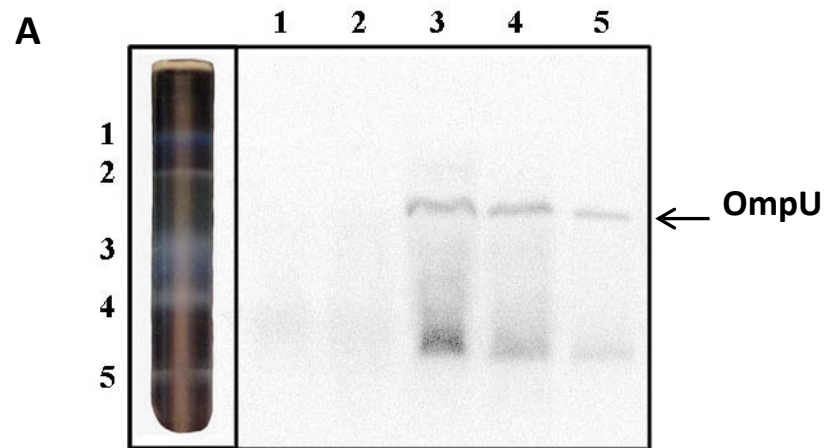
**Supplementary Data Fig. S3.** Illustrations of vesicle RNA (isolated from *V. cholerae* strain A1552) sequences aligned to chromosomal DNA sequences of *V. cholerae* N16961 strain. Red alignments are in the sense direction, blue alignments are in the anti-sense direction. \*: Height indicates the number of sequences aligned. **A.** Typical alignment view **B.** Region *vc2478 – vc2479* **C.** Region *vc0190 – vc0191* **D.** Region *vca0526 – vca0527*.

**Supplementary Data Fig. S4.** RNA extracted from an overnight culture of *Vibrio cholerae* O1 El Tor A1552 whole cells (*wc*) and vesicles (*v*) using the Total RNA Norgen kit. RNA from whole cells (5 µg) and RNA from vesicles (3.5µg) were run on a 12 % polyacrylamide denaturing gel stained in GelRed (panel A). The RNA was transferred to a membrane that was probed with probe *vc2478,5* (panel B) and consecutively re-probed first with probe *vc0190,5* (panel C) and then with probe *vca0526,5* (panel D).

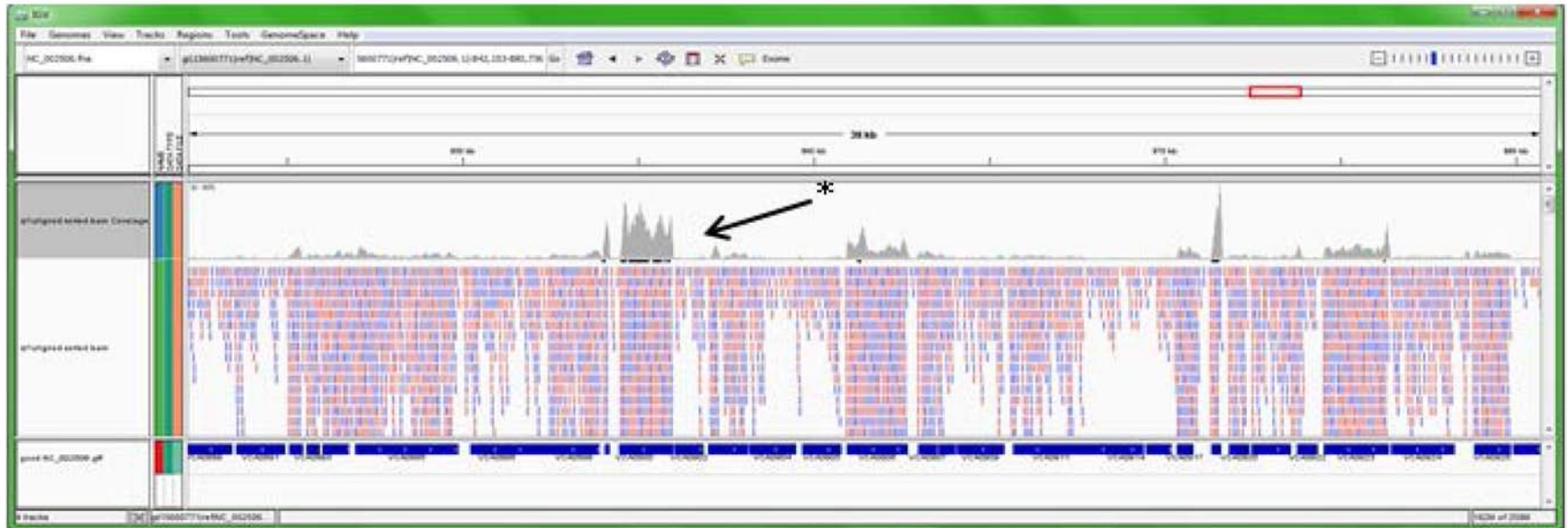
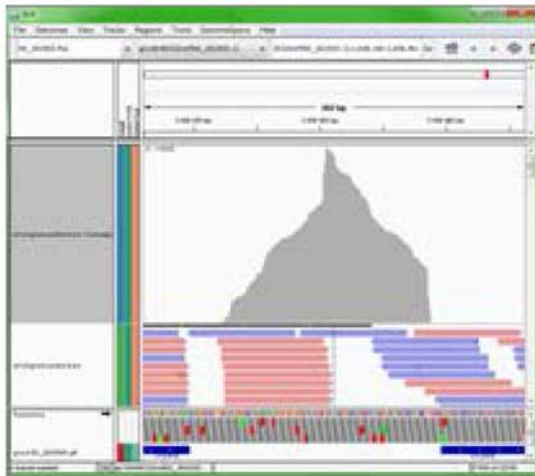
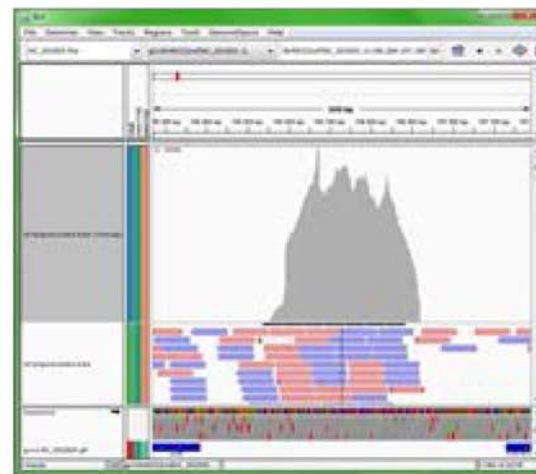
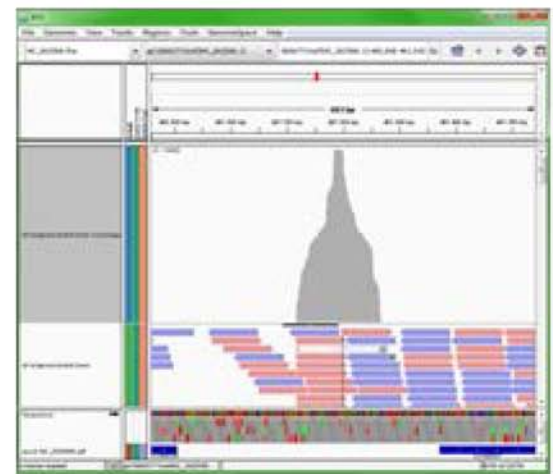
**Supplementary Data Fig. 5. DNase I treatment of RNA extracted from bacterial vesicles.** PCR analysis for detection of DNA in RNA samples extracted from vesicles released by *Vibrio cholerae* O1 El Tor strain A1552. The analysis was performed before (lanes 2 & 3) and after (lanes 4 & 5) treatment with DNase I. PCR products after amplifications using *ompU* primers (lanes 2 & 4) or *vc2478,5* primers (lanes 3 & 5) were analysed on a 1% agarose gel. Lane 1 shows a GeneRuler™ DNA Ladder (Fermentas).



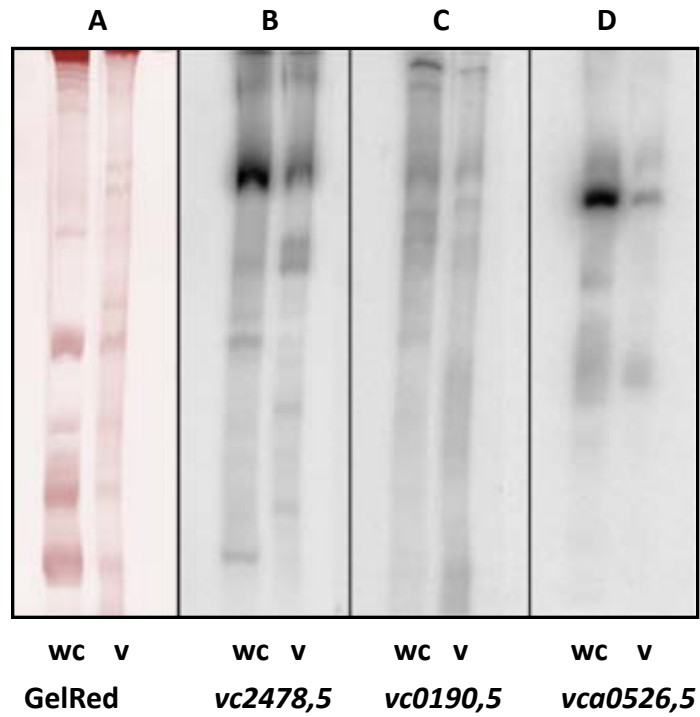
Supplementary Fig. S1



Supplementary Fig. S2

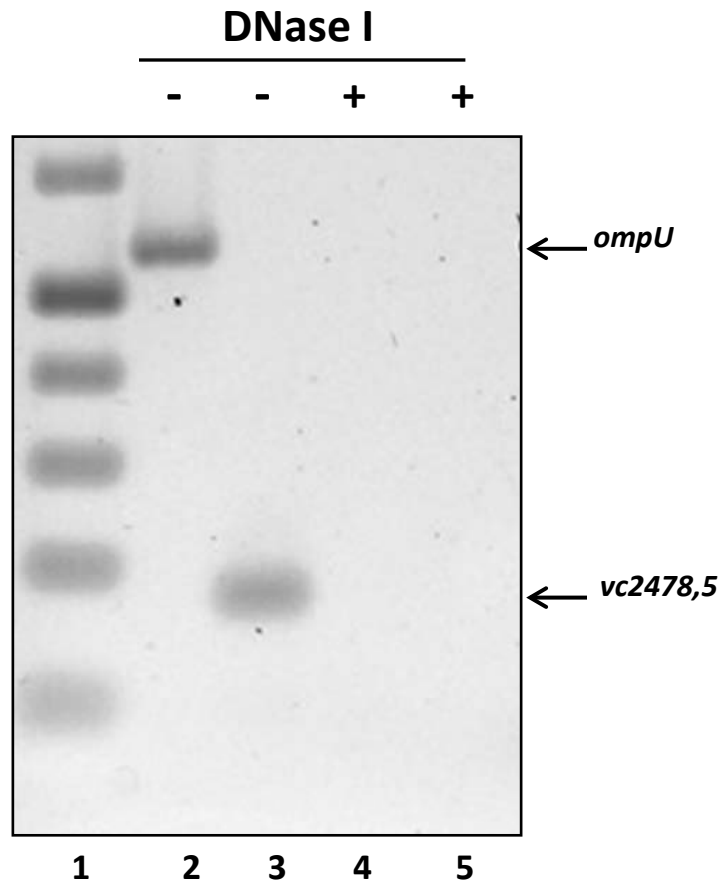
**a****b****c****d**

Supplementary Fig. S3



**Supplementary Fig. S4**





**Supplementary Fig. S5**

**Supplementary Table 1a.** Alignment of sequenced vesicle RNA to *V. cholerae* N16961 chromosome I.

Peak	Start	End	Region in N16961 chromosome I	
1090000	2658225	2658386	upstream of uncharacterized protein <b>vc2479</b>	6S RNA
120000	196545	196933	intergenic region between <b>vc0190</b> and <b>vc0191</b>	CsrB
111000	914315	914654	upstream of phage integrase family <b>vc0847</b>	SrsA
41500	943857	944200	uncharacterized protein <b>vc0883</b>	CsrB
41200	2375908	2376115	uncharacterized protein <b>vc2221</b>	
37800	2580714	2581110	upstream of uncharacterized protein <b>vc2410</b>	svch2581.1
23500	1588979	1589272	ribosome modulating factor <b>vc1484</b>	
17800	69300	69684	multidrug resistant protein <b>vc0069</b>	
13000	2334461	2334692	downstream of flagellin A <b>vc2188</b>	
12100	954751	954916	uncharacterized protein <b>vc0895</b>	GcvB
9400	36506	36828	uncharacterized protein <b>vc0038</b>	
7300	1578748	1578913	intergenic region between <b>vc1470</b> and <b>vc1471</b>	svch1579.1 & 2
7150	1584873	1585042	intergenic region between <b>vc1478</b> and <b>vc1479</b>	
6700	2619094	2619268	uncharacterized protein <b>vc2440</b>	
6200	381650	382992	elongation factor Tu-B <b>vc0362</b>	
5600	1719935	1720319	uncharacterized protein <b>vc1605a</b>	
5480	1992699	1993856	putative porin <b>vc1854</b>	
5100	1185676	1185924	putative glutaredoxin <b>vc1116</b>	
5000	335290	336538	elongation factor Tu-A <b>vc0321</b>	
4300	612554	613240	pseudo <b>vc0583</b>	svch613.1 & 2
4000	1300189	1300577	integration host factor subunit alfa <b>vc1222</b>	
3700	674802	675992	outer membrane protein <b>vc0633</b>	
3280	1471635	1471788	uncharacterized protein <b>vc1378</b>	
3100	2956240	2956682	ATP synthase FOF1 subunit I <b>vc2771</b>	
3010	2174106	2174453	acyl carrier protein <b>vc2020</b>	
2930	2332957	2334180	flagellin C <b>vc2187</b>	
2580	1318751	1319201	uncharacterized protein <b>vc1247</b>	
2280	2517106	2517539	autonomous glycyl radical cofactor <b>vc2361</b>	
2260	2365289	2366647	outer membrane protein <b>vc2213</b>	
2190	762615	762798	uncharacterized protein <b>vc0713</b>	
2000	1538123	1539689	cbb-3 type Cytochrome c oxidase, subunit II <b>vc1442</b>	
1980	1417217	1417374	upstream of uncharacterized protein <b>vc1333</b>	
1970	222697	222833	coenzyme A biosynthesis bifunctional protein <b>vc0215</b>	
1940	2063226	2063618	integration host factor subunit beta <b>vc1914</b>	
1910	2295417	2295764	flagellar capping protein <b>vc2140</b>	
1880	2754248	2755599	preprotein translocase subunit <b>vc2576</b>	
1800	1408994	1410076	galactoside ABC transporter, periplasmic D-galactose/D-glucose-binding protein <b>vc1325</b>	
1780	102446	102657	putative uncharacterized protein <b>vc0107</b>	RyhB & Spot_42
1750	1198128	1198673	DNA binding protein <b>vc1130</b>	
1690	1211912	1212296	cold shock-like protein <b>vc1142</b>	
1680	2295764	2296334	flagellar protein <b>vc2141</b>	
1650	2068862	2069279	DNA-binding protein HU-beta <b>vc1919</b>	

1650	2909984	2911682	Phosphoenolpyruvate carboxykinase [ATP] <b>vc2738</b>	
1600	1458356	1458653	uncharacterized protein <b>vc1368</b>	
1500	86471	87681	cytochrome peroxidase c551 <b>vc0089</b>	
1490	1670845	1673232	catalase/peroxidase <b>vc1560</b>	
1480	1794805	1795288	heat shock protein <b>vc1663</b>	
1470	2163539	2163748	uncharacterized protein <b>vc2009</b>	
1400	786571	787319	antioxidant ahp CTSA family protein <b>vc0731</b>	
1390	1536322	1537322	cbb-3 type Cytochrome c oxidase, subunit P <b>vc1439</b>	
1390	2949663	2950113	ATP synthase F0F1 subunit epsilon <b>vc2763</b>	
1370	2713024	2713375	RNA polymerase factor sigma-54 <b>vc2529</b>	
1330	1537495	1538123	cbb-3 type Cytochrome c oxidase, subunit II <b>vc1441</b>	
1330	2448193	2449428	Na(+)-translocating NADH-quinone reductase subunit <b>vc2294</b>	
1260	1537322	1537495	cbb-3 type Cytochrome c oxidase, subunit Q <b>vc1440</b>	
1260	2152257	2153283	glyceraldehyde-3-phosphate dehydrogenase <b>vc2000</b>	
1250	2249553	2250272	succinate dehydrogenase iron-sulfur subunit <b>vc2088</b>	
1240	580419	580787	upstream of and the whole carbon storage regulator <b>vc0548</b>	
1230	1644504	1644628	intergenic region between <b>vc1531</b> and <b>vc1532</b>	
1200	1097620	1099948	zink/cadmium/mercury/lead-transporting ATPase <b>vc1033</b>	
1190	2449428	2450823	Na(+)-translocating NADH-quinone reductase subunit A <b>vc2295</b>	
1180	2221154	2221925	flagellar biosynthesis sigma factor <b>vc2066</b>	
1140	1031609	1031884	phosphocarrier protein HPr <b>vc0966</b>	
1140	2394692	2394907	downstream of cytochrome c554 <b>vc2241</b>	
1140	2669986	2670098	upstream of 2-isopropylmalate synthase <b>vc2490</b>	svch2670.1
1120	1597539	1602466	uncharacterized protein <b>vc1492</b>	
1100	1029112	1029661	PTS system glucos-specific IIA component <b>vc0964</b>	
1100	2657848	2658199	uncharacterized protein <b>vc2478</b>	
1090	1838582	1838921	uncharacterized protein <b>vc1707</b>	
1070	564258	564667	lipoprotein <b>vc0533</b>	
1070	2152042	2152257	putative uncharacterized protein <b>vc1999</b>	
1070	2564959	2565969	cell division protein <b>vc2397</b>	
1060	2564178	2564959	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase <b>vc2396</b>	
1050	2166629	2168114	PTS system glucose-specific transporter subunit IIBC <b>vc2013</b>	
1020	2297656	2298854	flagellin D <b>vc2143</b>	
1000	2296334	2297482	flagellin B <b>vc2142</b>	
1000	462204	463183	malate dehydrogenase <b>vc0432</b>	
1000	1520426	1520674	intergenic region between <b>vc1422</b> and <b>vc1423</b>	svch1521.1
1000	1111516	1112882	long-chain fatty acid transport protein <b>vc1043</b>	
1000	756233	756588	sigma-54 modulation protein <b>vc0706</b>	
980	2253168	2254463	type II citrate synthase <b>vc2092</b>	
950	2420154	2420290	upstream of uncharacterized protein <b>vc2264</b>	
950	1310940	1311270	upstream of nicotinate-nucleotide--dimethylbenzimidazole phosphoribosyltransferase <b>vc1237</b>	
930	2654106	2654548	uncharacterized protein <b>vc2473</b>	
920	2019596	2019906	SpoVR family protein <b>vc1874</b>	
920	1447024	1447739	uncharacterized membrane protein <b>vc1358</b>	
910	2394907	2395221	cytochrome c554 <b>vc2241</b>	
910	1650724	1651517	uncharacterized protein <b>vc1539</b>	svch1651.1
890	2252361	2252772	succinate dehydrogenase cytochrome b556 subunit <b>vc2091</b>	

870	1214761	1215323	ATP-dependent Clp protease ATP-binding subunit <b>vc1144</b>	
870	1608509	1609145	solute/DNA competence effector, protein ProQ homolog <b>vc1497</b>	
870	76886	77365	ferritin <b>vc0078</b>	
860	305064	307130	acetyl-CoA synthetase <b>vc0298</b>	
850	2071844	2073125	ATP-dependent protease ATP-binding subunit <b>vc1921</b>	
830	2864880	2866417	aspartate ammonia-lyase <b>vc2698</b>	
830	278425	278694	DNA binding HU-alfa <b>vc0273</b>	
820	2444902	2446158	Na(+)-translocating NADH-quinone reductase subunit <b>vc2290</b>	
810	1021356	1022017	uncharacterized protein <b>vc0957</b>	
810	1814878	1815035	peptide ABC transporter substrate-binding protein <b>vc1680</b>	
810	2764786	2765042	upstream of <b>vc2597</b>	svch2765.1
800	1832388	1832689	uncharacterized protein <b>vc1699</b>	
800	76031	76651	universal stress protein A <b>vc0076</b>	
780	139954	140888	RNA polymerase sigma factor-32 <b>vc0150</b>	
770	2713375	2713710	sigma-54 modulation protein <b>vc2530</b>	
760	435431	435989	MSHA pilin protein <b>vc0409</b>	
740	1451138	1452295	amino acid ABC transporter substrate binding protein <b>vc1362</b>	
740	1158698	1160377	oligopeptide ABC transporter substrate binding protein <b>vc1091</b>	
730	633774	636488	bifunctional aconitate hydratase 2/2 methylisocitrate <b>vc0604</b>	
730	2081097	2082120	C4-dicarboxylate-binding periplasmic protein <b>vc1929</b>	
720	2834286	2834637	co-chaperonin, 10 kDa chaperonin 1 <b>vc2665</b>	
720	1135448	1135547	upstream of ArsR family transcriptional regulator <b>vc1068</b>	svch1136.1 & 2
720	2178616	2179044	uncharacterized protein <b>vc2026</b>	
700	2955395	2956240	ATP synthase F0F1 subunit A <b>vc2770</b>	
700	2447420	2448193	Na(+)-translocating NADH-quinone reductase subunit <b>vc2293</b>	
700	383504	384000	bactoferritin <b>vc0365</b>	
700	132719	133283	DPS family protein <b>vc0139</b>	
700	554239	554693	uncharacterized protein <b>vc0519</b>	
700	2014929	2016993	uncharacterized protein <b>vc1872</b>	
680	2954571	2955048	ATP synthase F0F1 subunit B <b>vc2768</b>	
680	2446782	2447420	Na(+)-translocating NADH-quinone reductase subunit <b>vc2292</b>	
670	692148	692445	polyribonucleotide nucleotidyltransferase <b>vc0647</b>	
670	2877100	2878819	putative Sodium/solute symporter <b>vc2705</b>	
650	2626588	2627926	enolase <b>vc2447</b>	
630	2832601	2834286	molecular chaperone <b>vc2664</b>	
630	1332776	1332950	DNA gyrase subunit A <b>vc1258</b>	
630	2203125	2203796	superoxide dismutase <b>vc2045</b>	svch2204.1
620	380939	381650	elongation factor G1 <b>vc0361</b>	
620	500283	500485	S-adenosylmethionine synthetase <b>vc0472</b>	
610	1319201	1320911	methyl-accepting chemotaxis protein <b>vc1248</b>	
600	2950113	2951534	ATP synthase F0F1 subunit beta <b>vc2764</b>	
600	2495392	2497494	elongation factor G 2 <b>vc2342</b>	
600	2501622	2501760	intergenic region inbetween <b>vc2344</b> and <b>vc2345</b>	
590	2250272	2252031	succinate dehydrogenase flavoprotein subunit <b>vc2089</b>	
590	2450823	2450982	upstream of Na(+)-translocating NADH-quinone reductase subunit <b>vc2295</b>	
590	2892438	2892948	uncharacterized protein <b>vc2717</b>	
580	2347213	2348056	chemotaxis protein methyltransferase 1 <b>vc2201</b>	

560	1374791	1375004	upsteam of phosphomethylpyrimidine kinase <b>vc1296</b>	<b>TPP riboswitch</b>
560	1321079	1321598	uncharacterized protein <b>vc1249</b>	
550	2344123	2345436	<b>flagellar hook protein vc2197</b>	
550	2187578	2190491	bifunctional acetaldehyde-CoA/alcohol dehydrogenase <b>vc2033</b>	
550	1283771	1284105	ds-DNA mimic protein <b>vc1208</b>	
540	1477000	1477430	uncharacterized protein <b>vc1384</b>	
540	2041347	2041496	intergenic region inbetween and parts of uncharacterized proteins <b>vc1893</b> and <b>vc1894</b>	
530	1523028	1524280	spermidine/putrescine ABC transporter, periplasmic spermidine/putrescine-binding protein <b>vc1425</b>	
530	1257042	1257620	uncharacterized protein <b>vc1183</b>	
520	1651869	1652043	uncharacterized protein <b>vc1539a</b>	
520	2952435	2954047	ATP synthase FOF1 subunit alfa <b>vc2766</b>	
520	623301	623784	DnaK suppressor protein <b>vc0596</b>	
520	2334684	2335901	<b>flagellin A vc2188</b>	
510	133961	134317	uncharacterized protein <b>vc0142</b>	
510	2083993	2084133	uncharacterized protein <b>vc1932</b>	
510	952618	952804	chemotaxis protein <b>vc0893</b>	
500	2955048	2955395	ATP synthase FOF1 subunit C <b>vc2769</b>	
500	2334692	2336031	<b>flagellin B vc2142</b>	
500	317733	318109	thioredoxin <b>vc0306</b>	
490	1300020	1300189	upstream of <b>vc1222</b>	
480	2327636	2327793	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase <b>vc2182</b>	
470	2582987	2584882	pyruvate dehydrogenase <b>vc2413</b>	
470	2951534	2952435	ATP synthase FOF1 subunit gamma <b>vc2765</b>	
470	2414371	2415329	elongation factor Ts <b>vc2259</b>	
470	2006853	2009215	formate acetyltransferase <b>vc1866</b>	
470	935777	935909	upstream of IS1004 transposase <b>vc0870</b>	
460	2782151	2782911	cAMP-regulatory protein <b>vc2614</b>	
460	107395	108097	cytochrome c4 <b>vc0112</b>	
450	2783856	2784067	N-succinylglutamate 5-semialdehyde dehydrogenase <b>vc2616</b>	
450	2954047	2954571	ATP synthase FOF1 subunit delta <b>vc2767</b>	
450	2856763	2857292	6-phosphofructokinase <b>vc2689</b>	
450	1976501	1977100	peptidoglycan-associated lipoprotein <b>vc1835</b>	
440	1385378	1386948	putative fumarate hydratase, class I <b>vc1304</b>	
440	2393494	2393849	nitrogen regulatory protein P-II <b>vc2239</b>	
430	1530646	1531594	putative stress protein <b>vc1433</b>	
415	2055506	2056621	alanine dehydrogenase <b>vc1905</b>	
410	1050496	1051173	Adenylate kinase <b>vc0986</b>	
410	77365	77729	universal stress protein B, ppGpp-dependent, membrane associated <b>vc0079</b>	
400	2348056	2349080	chemotaxis protein <b>vc2202</b>	
400	803130	803283	HesB family protein <b>vc0750</b>	
400	1100574	1101161	uridine phosphorylase <b>vc1034</b>	
390	354674	355141	2,3-bisphosphoglycerate-independent phosphoglycerate mutase <b>vc0336</b>	
390	2130297	2130520	putative aspartate amino transferase <b>vc1977</b>	
390	302338	302528	Acetyl-CoA carboxylase, biotin carboxylase <b>vc0295</b>	
390	2513905	2514095	uncharacterized protein <b>vc2357</b>	
380	2218550	2219998	chemotaxis protein <b>vc2063</b>	
380	2610643	2611874	outer membrane channel protein <b>vc2436</b>	

370	2944713	2945721	3-ketoacyl-CoA thiolase <b>vc2759</b>	
370	2343586	2343966	flagellar basal-body rod protein <b>vc2196</b>	
370	1801911	1802093	intergenic region between <b>vc1669</b> and <b>vc1670</b>	
350	564667	565745	RNA polymerase sigma factor-38 <b>vc0534</b>	
350	348760	349396	anti-RNA polymerase sigma 70 factor <b>vc0330</b>	
350	96097	97811	methyl accepting chemotaxis protein <b>vc0098</b>	
340	2581316	2582415	dihydrolipoyl dehydrogenase <b>vc2412</b>	
340	2446158	2446782	Na(+)-translocating NADH-quinone reductase subunit <b>vc2291</b>	
330	2524608	2525234	aerobic respiration control protein <b>vc2368</b>	
330	2345436	2346180	basal-body rod modification protein <b>vc2198</b>	
330	1407889	1408268	uncharacterized proteins <b>vc1322</b> and <b>vc1323</b>	
330	2143461	2143732	uncharacterized protein <b>vc1991</b>	
320	2584882	2587569	pyruvate dehydrogenase E1 component <b>vc2414</b>	
320	792153	792676	putative Acetoin utilization protein <b>vc0737</b>	
320	1381458	1382755	serine transporter <b>vc1301</b>	
320	1396268	1396793	methyl-accepting chemotaxis protein <b>vc1313</b>	
320	2799112	2799302	DamX-related protein <b>vc2627</b>	
310	2214140	2214592	purine-binding chemotaxis protein <b>vc2059</b>	
310	1336667	1336810	uncharacterized protein <b>vc1262</b>	
300	2220725	2221154	chemotaxis protein <b>vc2065</b>	
300	1021344	1021482	intergenic region between <b>vc0956</b> and <b>vc0957</b>	
290	2263005	2263276	intergenic region between <b>vc2105</b> and <b>vc2106</b>	
280	1114605	1114725	intergenic region between <b>vc1045</b> and <b>vc1046</b>	
280	1061324	1062905	PTS systemN-acetylglucosamine-specific transporter subunit <b>vc0995</b>	
280	2801124	2801281	fimbrial assembly protein <b>vc2630</b>	
280	132624	132718	uncharacterized protein <b>vc0138</b>	
270	665838	666197	essential respiratory protein A, iron-sulfur insertion prot <b>vc0627</b>	
270	2346618	2347031	flagellar basal body rod protein <b>vc2200</b>	
270	2221925	2222617	site-determining protein <b>vc2067</b>	
260	2587569	2588098	pyruvate dehydrogenase complex repressor <b>vc2415</b>	
260	53506	53590	uncharacterized protein <b>vc0059</b>	
250	2349799	2350245	negative regulator of flagellin synthesis <b>vc2204</b>	
250	2288320	2288626	flagellar hook-basal body complex protein <b>vc2134</b>	
250	2627926	2628183	CTP synthase <b>vc2448</b>	
250	422634	423163	single-stranded DNA-binding protein <b>vc0397</b>	
250	2420288	2420723	UPF0325 protein <b>vc2264</b>	
250	2252772	2252962	upstream of <b>vc2091</b>	
250	937693	937804	uncharacterized protein <b>vc0874</b>	
240	2549464	2549770	upstream of RNA-directed DNA polymerase <b>vc2385</b>	<b>IGR3</b>
230	2839602	2840135	regulator of ribonuclease activity A <b>vc2672</b>	
220	1401314	1402103	outer membrane protein <b>vc1318</b>	

**Peak:** the highest number of RNA sequences aligned to a specific nucleotide the region. **Grey box:** region contains a sRNA. **Start** and **end:** positions in the chromosome. **Orange box:** cytoplasmic location, **Blue box:**

inner membrane location, **Red box**: outer membrane location, **Purple box**: membrane, **Light yellow box**: pilus association, **Yellow box**: flagellar association, and **White box**: unknown location.

**Supplementary Table 1b.** Alignment of sequenced vesicle RNA to *V. cholerae* N16961 chromosome II.

Peak	Start	End	Region in N16961 chromosome II	
115300	461118	461267	intergenic region between <b>vca0526</b> and <b>vca0527</b>	svch462.1
92300	783813	784124	uncharacterized protein <b>vca0839</b>	CsrB
24200	886914	887278	uncharacterized protein <b>vca0935</b>	
19400	66495	66837	major outer membrane lipoprotein <b>vca0059</b>	
8400	61091	61300	uncharacterized protein <b>vca0052</b>	
7590	1031448	1031574	upstream of LuxR family transcriptional regulator <b>vca1078</b>	
7500	308592	309077	translation initiation factor IF-3 <b>vca0288</b>	LR-PK1
7000	485524	485763	uncharacterized protein <b>vca0547</b>	svch486.1
5800	213120	213285	upstream of GMP reductase <b>vca0197</b>	svch214.1
4650	893294	893459	downstream of maltose transport system permease protein <b>vca0943</b>	MicX
4220	817334	819261	hemagglutinin/protease <b>vca0865</b>	
3090	819602	820290	outer membrane protein <b>vca0867</b>	
2700	284969	285940	methyl-accepting chemotaxis protein <b>vca0268</b>	
2180	627734	628099	uncharacterized protein <b>vca0689</b>	
2180	893459	893603	maltose transport system permease protein <b>vca0943</b>	svch894.2
1670	834562	835685	uncharacterized protein <b>vca0883</b>	
1600	4061	4387	uncharacterized protein <b>vca0004</b>	
1430	308245	308592	upstream of translation initiation factor IF-3 <b>vca0288</b>	
1370	487754	488020	uncharacterized protein <b>vca0551</b>	
1140	203421	203797	cold shock DNA-binding domain-containing protein <b>vca0184</b>	
1100	39233	40071	upstream of and uncharacterized protein <b>vca0032</b>	
1020	232124	232329	upstream of uncharacterized protein <b>vca0213</b>	svch233.1
1010	994761	994875	upstream of PTS system mannitol-specific EIICBA component <b>vca1045</b>	Mds
950	88629	89022	uncharacterized protein <b>vca0078</b>	
830	776851	776956	uncharacterized protein <b>vca0831</b>	
660	629320	630236	acetyl-CoA reductase <b>vca0691</b>	
630	833061	833490	uncharacterized protein <b>vca0881</b>	
610	628099	629320	acetyl-CoA acetyltransferase <b>vca0690</b>	
580	971762	972073	uncharacterized protein <b>vca1024</b>	
560	532373	532660	hemolysin <b>vca0594</b>	
550	237171	237395	upstream of hemolysin <b>vca0219</b>	
530	220268	220397	pseudo <b>vca0203</b>	
510	684347	685544	uncharacterized protein <b>vca0738</b>	
500	508610	509011	uncharacterized protein <b>vca0571</b>	
500	871320	871655	uncharacterized protein <b>vca0919</b>	
500	929290	929858	uncharacterized protein <b>vca0981</b>	
490	1003717	1004369	uncharacterized protein <b>vca1054</b>	
480	546334	546515	uncharacterized protein <b>vca0609</b>	
450	556852	558082	upstream of and transaldolase <b>vca0623</b>	svch557.1
430	702687	702937	arginine ABC transporter, permease protein <b>vca0758</b>	
420	833490	834562	uncharacterized protein <b>vca0882</b>	
410	678373	678819	uncharacterized protein <b>vca0732</b>	
400	89052	90407	uncharacterized protein <b>vca0079</b>	
400	204597	205022	uncharacterized protein <b>vca0186</b>	
400	760422	760987	agmatinase <b>vca0814</b>	



390	292709	292829	upstream of IS1004 transposase <b>vca0275</b>	
390	854501	855376	uncharacterized protein <b>vca0900</b>	
370	38714	39233	methyl-accepting chemotaxis protein <b>vca0031</b>	
370	508251	508610	translation initiation factor Sui1 <b>vca0570</b>	
360	1050275	1050461	chemotaxis protein <b>vca1095</b>	
360	1052862	1053168	uncharacterized protein <b>vca1097</b>	
350	1068928	1069971	spermidine/putrescine ABC transporter, periplasmic spermidine/putrescine-binding protein <b>vca1113</b>	
340	1029148	1029441	uncharacterized protein <b>vca1075</b>	
330	713202	713355	uncharacterized protein <b>vca0769</b>	
320	42217	43058	uncharacterized protein <b>vca0034</b>	
320	760210	760373	intergenic region between <b>vca0813</b> and <b>vca0814</b>	
320	601944	602091	probable anaerobic C4-dicarboxylate transporter <b>vca0665</b>	
310	787274	787400	downstream of glyceraldehyde 3-phosphate dehydrogenase <b>vca0844</b>	
310	86599	86766	sulfate permease family protein <b>vca0077</b>	
300	471351	471626	Sec-independent protein translocase protein <b>vca0533</b>	
300	1068580	1068928	tRNA(Met) cytidine acetyltransferase <b>vca1112</b>	
290	295897	296280	glycine cleavage system H protein <b>vca0277</b>	
290	855376	856001	uncharacterized protein <b>vca0901</b>	
280	184104	184447	cold shock transcriptional regulator <b>vca0166</b>	
280	483866	484022	upstream of uncharacterized protein <b>vca0546</b>	svch484.1
280	750895	751260	uncharacterized protein <b>vca0806</b>	
280	1049376	1049696	purine-binding chemotaxis protein <b>vca1093</b>	
270	1026467	1026730	bifunctional proline dehydrogenase/pyrroline-5-carboxylate dehydrogenase <b>vca1073</b>	
270	637183	637351	upstream of Glucose-1-phosphate adenylyltransferase 2 <b>vca0699</b>	
260	993053	993422	Ccm2-related protein <b>vca1042</b>	
260	897542	897683	intergenic region between <b>vca0945</b> and <b>vca0946</b>	
260	969225	969635	uncharacterized protein <b>vca1021</b>	
250	854000	854194	uncharacterized protein <b>vca0899</b>	
240	194828	195055	uncharacterized protein <b>vca0177</b>	
240	531212	532265	uncharacterized protein <b>vca0593</b>	
240	1021264	1021692	sodium/proline symporter <b>vca1071</b>	
240	1022765	1023456	uncharacterized protein <b>vca1072</b>	
230	333994	334479	uncharacterized protein <b>vca0332</b>	
230	580379	580807	uncharacterized protein <b>vca0645</b>	
230	876133	876422	methyl-accepting chemotaxis protein <b>vca0923</b>	
220	860933	861670	methyl-accepting chemotaxis protein <b>vca0906</b>	
220	832592	833042	uncharacterized protein <b>vca0880</b>	
210	180156	180453	upstream of tryptophanase <b>vca0161</b>	

**Peak:** the highest number of RNA sequences aligned to a specific nucleotide the region. **Grey box:** region contains a sRNA. **Start and end:** positions in the chromosome. **Orange box:** cytoplasmic location, **Red box:** outer membrane location, **Blue box:** inner membrane location, **Light blue box:** periplasmic location, **Purple box:** membrane, **Green box:** secreted protein, and **White box:** unknown location.



## Supplemental References

- Amako, K., Meno, Y., and Takade, A. (1988). Fine structures of the capsules of *Klebsiella pneumoniae* and *Escherichia coli* K1. *J. Bacteriol.* *170*, 4960–4962.
- Benson, S.A., and Decloux, A. (1985). Isolation and characterization of outer membrane permeability mutants in *Escherichia coli* K-12. *J. Bacteriol.* *161*, 361–367.
- Bolivar, F., Rodriguez, R.L., Greene, P.J., Betlach, M.C., Heyneker, H.L., Boyer, H.W., Crosa, J.H., and Falkow, S. (1977). Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. *Gene* *2*: 95–113.
- Casadaban, M.J. (1976). Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. *J. Mol. Biol.* *104*, 541–555.
- Chatterjee, S., Mondal, A.K., Begum, N.A., Roychoudhury, S., and Das, J. (1998). Ordered cloned DNA map of the genome of *Vibrio cholerae* 569B and localization of genetic markers. *J. Bacteriol.* *180*, 901–908.
- Furste, J.P., Pansegrau, W., Frank, R., Blocker, H., Scholz, P., Bagdasarian, M., and Lanka, E. (1986). Molecular cloning of the plasmid RP4 primase region in a multi-host-range *tacP* expression vector. *Gene*. *48*, 119–31.
- Guzman, C.A., Piatti, G., Staendner, L.H., Biavasco, F., and Pruzzo, C. (1995) Export of *Bordetella pertussis* serotype 2 and 3 fimbrial subunits by *Escherichia coli*. *FEMS Microbiol. Lett.* *128*, 189–194.
- Miller, V.L., Taylor, R.K., and Mekalanos, J.J. (1987). Cholera toxin transcriptional activator *toxR* is a transmembrane DNA binding protein. *Cell* *48*, 271–279.
- Taylor, R.K., Miller, V.L., Furlong, D.B., and Mekalanos, J.J. (1987). Use of *phoA* gene fusions to identify a pilus colonization factor co-coordinately regulated with cholera toxin. *Proc. Natl. Acad. Sci. USA* *84*, 2833–2837.
- Thelin, K.H., and Taylor, R.K. (1996). Toxin-coregulated pilus, but not mannose-sensitive hemagglutinin, is required for colonization by *Vibrio cholerae* O1 El Tor biotype and O139 strains. *Infect. Immun.* *64*, 2853–2856.
- Yildiz, F.H., and Schoolnik, G.K. (1998). Role of *rpoS* in stress survival and virulence of *Vibrio cholerae*. *J. Bacteriol.* *180*, 773–784.