

Table S2. Secreted protein candidates and results of manual analysis

GI	JUDGMENT	N1	N2	EVIDENCE
254780384	no SP, extracellular	0	0	1. Type I Secretion System substrate; 2. export by type I secretion system
254780523	No SP, extracellular	0	1	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780685	No SP, outermembrane	0	0	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780380	No SP, periplasmic	0	0	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780381	No SP, periplasmic	0	0	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780379	No SP, periplasmic	0	0	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780378	No SP, extracellular	0	1	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780520	No SP, extracellular	0	0	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780524	No SP, extracellular	0	0	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780531	No SP, extracellular	0	0	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780525	No SP, extracellular	0	0	1. flagellar component; 2. do not have SP export by flagellar assembly machinery
254780733	No SP, periplasmic or extracellular	6	0	1. flp, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
254780732	No SP, periplasmic or extracellular	6	0	1. flp, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
254780730	No SP, periplasmic or extracellular	5	1	1. flp, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
254780736	No SP, periplasmic or extracellular	6	0	1. flp, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
254780734	No SP, periplasmic or extracellular	6	0	1. flp, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
254781110	No SP, periplasmic or extracellular	6	0	1. TadE or Tad F, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
254780934	No SP, periplasmic or extracellular	6	0	1. TadE or Tad F, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
254780833	No SP, periplasmic or extracellular	6	1	1. TadE or Tad F, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
254780388	No SP, periplasmic or extracellular	6	0	1. TadE or Tad F, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
254781108	No SP, periplasmic or extracellular	5	2	1. TadE or Tad F, pilus assembly protein; 2. has TMH but can be processed by TadV to become periplasmic. Do not have SP
255764485	SP, outermembrane	0	0	1. CpaD, pilus assembly; 2. orthologs proved by Phobius and one mode of SignalP; 3. start point is wrong, should remove "MMVEYMITILFGGVCFKGLANMRSLSISCLKTIFWKNFFLRTL", then Phobius and one mode of SignalP predict SP
254780707	SP, extracytoplasmic	3	1	1. Function; 2. ortholog proved by both modes of SignalP and Phobius; 3. COG suggest this protein is usually periplasmic
254780396	SP, extracytoplasmic	6	1	1. function; 2. ortholog proved by both modes of SignalP and Phobius; 3. pfam suggest this protein contains domain that is usually periplasmic
254780698	SP, extracytoplasmic	4	1	1. function; 2. pfam suggest this protein contains domain that is usually periplasmic; 3. start point wrong, should remove "MLQKKIQSSAKKTFLTYPYGSIYIQRNIQL", then 4 program predict SPs
254780930	SP, extracytoplasmic	3	1	1. function; 2. ortholog proved by both modes of SignalP and Phobius
254780700	SP, extracytoplasmic?	6	1	1. ortholog proved by both modes of SignalP and Phobius; 2. COG suggest this protein is usually periplasmic
254780772	SP, extracytoplasmic?	6	1	1. function; 2. start point is wrong, should remove "MHKSTEDFRRIRLLEKYFPRSFQ", then phobius will also predict SP
254781112	SP, extracytoplasmic?	6	1	1. function; 2. ortholog can be verified by both modes of SignalP and Phobius
254780764	SP, extracytoplasmic?	6	1	1. function; 2. Ortholog; 3. start point is wrong, should remove "MFTHAEKILYS LDLRKY", then both phibus and signalP predict it as signal protien
254781174	SP, extracytoplasmic?	6	1	1. function; 2. ortholog
254780561	SP, extracytoplasmic	4	2	Function
254780314	SP, extracytoplasmic	0	2	1. function; 2. ortholog proved by two modes of SignalP and Phobius
254780635	SP, extracytoplasmic	0	2	ortholog proved by one mode of SignalP and Phobius
254780350	SP, extracytoplasmic	4	2	ortholog proved by one mode of SignalP and Phobius, at least periplasm
254780953	SP, extracytoplasmic	0	2	1. function; 2. ortholog proved by one mode of SignalP and Phobius
254780898	SP, extracytoplasmic	6	2	1. function; 2. ortholog proved by one mode of SignalP and MEMSAT
254780395	SP, extracytoplasmic	2	2	1. function; 2. ortholog proved by two modes of SignalP and Phobius
254780798	SP, extracytoplasmic	5	2	1. function; 2. ortholog confirmed by two modes of SignalP
254780728	SP, extracytoplasmic	5	2	Function
254780592	SP, extracytoplasmic	4	2	Function
254780376	SP, extracytoplasmic	2	2	1. function, although it is Flagellar assembly protein, but this one is probably transported by Sec into periplasm to set up the flagellar machinery, it is FlgI, and it is

				an exception; 2. ortholog, confirmed by two modes of signalP too
254780477	SP, extracytoplasmic	5	2	ortholog proved by one mode of SignalP and Phobius
254780745	SP, extracytoplasmic	3	2	1. function; 2. pfam suggest this protein contains domain that is usually periplasmic
254781221	SP, extracytoplasmic	0	2	ortholog predicted to have SP
254781045	SP, extracytoplasmic	0	2	1. function; 2. ortholog, confirmed by two modes of signalP and phobius too
254780221	SP, extracytoplasmic	0	3	
254780528	SP, extracytoplasmic	0	3	Function
254780959	SP, extracytoplasmic	5	3	
254780954	SP, extracytoplasmic	5	3	
254781078	SP, extracytoplasmic	4	3	
254780556	SP, extracytoplasmic	2	3	
254780906	SP, extracytoplasmic	0	3	
254780737	SP, extracytoplasmic	0	3	
254780735	SP, extracytoplasmic	4	3	a bit weird, as it is flp homolog
254780281	SP, extracytoplasmic	5	3	Ortholog
254780342	SP, extracytoplasmic	2	3	Function
254780984	SP, extracytoplasmic	0	3	Close homolog is 254780981, which is predicted to have SP by 2 methods out of 4
254780935	SP, extracytoplasmic	4	3	Function
254780547	SP, extracytoplasmic	2	3	Function
254781014	SP, extracytoplasmic	4	3	
254781128	SP, extracytoplasmic	3	3	
254780759	SP, extracytoplasmic	0	3	
254781010	SP, extracytoplasmic	0	3	
254780377	SP, extracytoplasmic	3	3	function, although it is Flagellar assembly protein, but this one is probably transported by Sec into periplasm, it is FlgA, and it is an exception
254780375	SP, extracytoplasmic	0	3	
254780374	SP, extracytoplasmic	2	3	function, although it is Flagellar assembly protein, but this one is probably transported by Sec into periplasm, it is FlgH, and it is an exception
254780717	SP, extracytoplasmic	4	3	function
254780435	SP, extracytoplasmic	3	3	function
254780965	SP, extracytoplasmic	5	3	
254780747	SP, extracytoplasmic	6	3	1. Function; 2. structure prediction, the TMH predicted in the middle of the protein is not true judging from the structure template
254780207	SP, extracytoplasmic	0	3	
254780141	SP, extracytoplasmic	3	3	function
254780589	SP, extracytoplasmic	2	3	
254780563	SP, extracytoplasmic	0	4	function
254780951	SP, extracytoplasmic	3	4	
254780170	SP, extracytoplasmic	4	4	function
254780308	SP, extracytoplasmic	4	4	function
254780907	SP, extracytoplasmic	0	4	
254780909	SP, extracytoplasmic	0	4	
254781156	SP, extracytoplasmic	0	4	
254781157	SP, extracytoplasmic	0	4	
254780607	SP, extracytoplasmic	0	4	
254780199	SP, extracytoplasmic	5	4	function
254781207	SP, extracytoplasmic	5	4	ortholog
254780542	SP, extracytoplasmic	0	4	
254780548	SP, extracytoplasmic	5	4	function
254781121	SP, extracytoplasmic	0	4	
254780209	SP, extracytoplasmic	3	4	
254780727	SP, extracytoplasmic	3	4	function
254780598	SP, extracytoplasmic	0	4	function
254780844	SP, extracytoplasmic	4	4	
254780537	SP, extracytoplasmic	2	4	function
254781003	SP, extracytoplasmic	3	4	
254780443	SP, extracytoplasmic	4	4	
254780981	SP, extracytoplasmic	1	2	Close homolog is 254780984, which is predicted to have SP by 3 methods out of 4
254780220	SP, extracytoplasmic	0	2	
255764500	SP, extracytoplasmic	2	2	
254781159	SP, extracytoplasmic	0	2	
254781005	SP, extracytoplasmic	0	2	
254780886	SP, extracytoplasmic	3	2	
254780963	SP, extracytoplasmic	3	2	
254780914	SP, extracytoplasmic?	6	2	
254780135	SP, extracytoplasmic?	6	2	

254780929	SP, extracytoplasmic?	6	2	
254781007	SP, extracytoplasmic?	6	2	
254780980	SP, extracytoplasmic	0	1	close homolog is 254780556, which is predicted to have SP by 3 out of 4 methods
254780311	cytoplasmic	0	1	1. function as cytoplasmic enzyme; 2. structure prediction
254780313	cytoplasmic	0	1	1. function, work with ribosome; 2. structure prediction
254780315	cytoplasmic	0	1	
254781149	cytoplasmic	0	1	Function
254781058	cytoplasmic	0	1	1. function, this is the C-terminal part of phage lysozyme; 2. structure prediction
254781197	cytoplasmic	0	1	Function
254781071	cytoplasmic	0	1	1. this is just the middle piece of a protein; 2. structure prediction
254780392	cytoplasmic	0	1	1. Function; 2. structure prediction
254780482	cytoplasmic	0	1	1. function; 2. structure prediction
255764495	cytoplasmic	0	1	ortholog cannot be verified by either mode of signalP or Phobius
255764462	cytoplasmic	0	1	function
254780908	cytoplasmic	0	1	
254780918	cytoplasmic	0	1	
254780912	cytoplasmic	0	1	
254781026	cytoplasmic	0	1	1. function; 2. this is the middle part of a big protein; 3. structure prediction
254780195	cytoplasmic	2	1	1. function; 2. structure prediction
254780942	cytoplasmic	0	1	function
254781205	cytoplasmic	0	1	
254781162	cytoplasmic	0	1	function
254780168	cytoplasmic	0	1	1. function; 2. structure prediction; 3. ortholog proved by one mode of SignalP and Phobius
254780164	cytoplasmic	0	1	Function
254780768	cytoplasmic	0	1	1. Ortholog; 2. pfam
254780640	cytoplasmic	0	1	Function
254780543	cytoplasmic	0	1	Function
254780227	cytoplasmic	3	1	function as metabolic enzyme
254780724	cytoplasmic	0	1	Function
254780470	cytoplasmic	0	1	1. function; 2. structure prediction
254780920	cytoplasmic	2	1	Function
peg_1087	cytoplasmic	0	1	
254780132	cytoplasmic	0	1	Function
254780156	cytoplasmic	0	1	Function
254781097	cytoplasmic	5	1	1. structure prediction; 2. tiger say this step occur in cytoplasm
254780154	cytoplasmic	0	1	
254781099	cytoplasmic	0	1	1. structure prediction; 2. ortholog can only be verified by Phobius; 3. PRK says this step is cytoplasmic
254780787	cytoplasmic	0	1	function, translation initiation factor IF-2
254781036	cytoplasmic	0	1	1. function; 2. pfam
254781031	cytoplasmic	2	1	1. Function; 2. structure prediction
254780439	cytoplasmic	2	1	1. function; 2. pfam
254780434	cytoplasmic	0	1	1. function; 2. pfam
peg_473	cytoplasmic	0	1	function
254780322	cytoplasmic	0	1	1. function; 2. structure prediction
254780742	cytoplasmic	0	1	1. function; 2. pfam
254780219	cytoplasmic	0	1	
254780261	cytoplasmic	0	1	function, ribosome protein
254780266	cytoplasmic	0	1	function, ribosome protein
254780361	cytoplasmic	0	1	function, ribosome protein
254780687	cytoplasmic	0	1	Function, flagellar cytoplasmic component
254780682	cytoplasmic?	0	1	
254780510	cytoplasmic?	0	1	
254781039	cytoplasmic	3	2	function as metabolic enzyme
peg_887	cytoplasmic	0	2	function in tRNA processing
254781015	cytoplasmic	0	2	this does not look like a complete protein, ortholog is a Multi-TMHs protein, but this one lost most of the transmembrane part.
peg_1064	cytoplasmic	0	2	ortholog not predicted to have SP by both modes of SignalP and Phobius
254781048	cytoplasmic	3	2	function in TCA cycle
254780319	cytoplasmic	0	3	function, ribosome protein
255764476	transmembrane, SP not likely real	6	1	1. function; 2. multi-TMHs
255764499	transmembrane	6	1	1. Single-TMH, major part cytoplasmic 2. ortholog cannot be verified by either mode of signalP or phobius
254780140	Transmembrane, SP not likely true	6	1	1. single-TMH, major part periplasmic 2. ortholog can be proved by only one mode of signalp

254780519	transmembrane, SP not likely real	6	1	1. Function, flagellar transmembrane component; 2. multi-TMHs
254780695	transmembrane, SP not likely real	6	1	1. Function, flagellar transmembrane component; 2. double-TMH
255764488	transmembrane, SP not likely real	6	1	1. Function, flagellar transmembrane component; 2. double-TMH
254780507	transmembrane, SP not likely true	6	1	1. Function, flagellar transmembrane component; 2. Single-TMH, major part cytoplasmic
254780545	transmembrane, but SP not likely real	6	1	Single-TMH, major part cytoplasmic
254780752	transmembrane, SP not likely real	6	1	1. single-TMH, major part periplasmic; 2. ortholog
254780577	transmembrane, SP not likely real	6	1	1. function; 2. single-TMH, major part periplasmic
254780623	transmembrane, SP not likely real	6	1	1. Single-TMH at C-terminal, major part periplasmic; 2. ortholog
254780436	transmembrane, SP not likely real	4	1	single-TMH, major part periplasmic
254780961	transmembrane, SP not likely real?	6	1	1. single-TMH, major part periplasmic; 2. ortholog do not have SP by neither mode of SignalP or Phobius; 3. start point is wrong, should add "MSFNVRNFVLWI" in front, and it has one SP, one TMH
255764508	transmembrane, SP might be right	6	1	double-TMH
254780367	transmembrane, SP not likely real	6	1	multi-TMHs
254780866	transmembrane, SP not likely real	6	1	multi-TMHs
254780568	transmembrane, SP not likely real	6	1	multi-TMHs
255764466	transmembrane, SP not likely real	6	1	multi-TMHs
254780402	transmembrane, SP not likely real	6	1	double-TMH
254780228	transmembrane, SP not likely real	6	1	1. double-TMHs; 2. start wrong, should add "MMLILFI", then it has double TMHs
254780852	transmembrane, SP not likely real	6	1	multi-TMHs
254780917	transmembrane, SP not likely real	6	1	1. function; 2. multi-TMHs
254780708	transmembrane, SP not likely real	6	1	multi-TMHs
254780705	transmembrane, SP not likely real	6	1	1. function; 2. multi-TMHs
254781033	transmembrane, SP not likely real	6	1	multi-TMHs
254780450	transmembrane, SP not likely real	3	1	double-TMH
254780774	transmembrane, SP not likely real	6	1	multi-TMHs
254781079	transmembrane, SP not likely real	5	1	multi-TMHs
254780793	transmembrane, SP not likely real	6	1	multi-TMHs
254780171	transmembrane, SP not likely real	6	1	1. function; 2. multi-TMHs
254780483	transmembrane, SP not likely real	6	1	1. function; 2. multi-TMHs
255764497	transmembrane, SP not likely real	6	1	multi-TMHs
255764468	transmembrane, SP not likely real	6	1	1. multi-TMHs; 2. start point is wrong, should add "MSRKVRKFDTVFPFTNYI RYSIISLPVVFLGVVCCLLAILIIFKDSLNSISFGLFG" in front
254780860	transmembrane, SP not likely real	6	1	multi-TMHs
255764512	transmembrane, SP not likely real	6	1	1. Single-TMH, major part cytoplasmic 2. ortholog cannot be verified by either mode of signalP or phobius
254781169	transmembrane, SP not likely real	6	1	multi-TMHs
254780799	transmembrane, SP not likely real	6	1	multi-TMHs
254780425	transmembrane, SP not likely real	6	1	Multi-TMHs
254780494	transmembrane, SP not likely real	6	1	1. function; 2. Multi-TMHs
254780335	transmembrane, SP not likely real	6	1	Multi-TMHs
254780721	transmembrane, SP not likely real	6	1	Multi-TMHs
254780298	transmembrane, SP not likely real	6	1	1. function; 2. Multi-TMHs
254780291	transmembrane, SP not likely real	6	1	1. Single-TMH, major part periplasmic 2. ortholog predicted to have SP by only one mode of SignalP
254780593	transmembrane, SP not likely real	6	1	Multi-TMHs
254780576	transmembrane, SP not likely real	6	1	1. function; 2. Multi-TMHs
254780841	transmembrane, SP not likely real	6	1	Multi-TMHs
254780924	transmembrane, SP not likely real	6	1	Multi-TMHs
254780925	transmembrane, SP not likely real	6	1	1. function; 2. Multi-TMHs
254781178	transmembrane, SP not likely real	6	1	1. Multi-TMHs; 2. Ortholog
254780719	transmembrane, SP not likely real	6	1	1. function; 2. Multi-TMHs
254781002	transmembrane, SP not likely real	6	1	Multi-TMHs
254780323	transmembrane, SP not likely real	5	1	1. function; 2. multi-TMHs
254780967	transmembrane, SP not likely real	6	1	Multi-TMHs
254780815	transmembrane, SP not likely real	5	1	1. Single-TMH, major part cytoplasmic; 2. Pfam; 3. ortholog predicted to have SP only by Phobius
254780749	transmembrane, SP not likely real	6	1	Multi-TMHs
254781189	transmembrane, SP not likely real	6	1	Multi-TMHs
254781042	transmembrane, SP not likely real	6	1	Multi-TMHs
254780558	transmembrane, SP not likely real	6	1	1. function; 2. Pfam; 3. single TMH at N-terminus and mainly periplasmic,
254781158	transmembrane, SP not likely real	6	1	1. single TMH at N-terminus and mainly periplasmic; 2. ortholog has SP by Phobius and one mode of SignalP
254780421	transmembrane, SP not likely real	6	1	single TMH at N-terminus and mainly periplasmic,
254780386	transmembrane, SP not likely real	5	1	single TMH at N-terminus and mainly periplasmic,

254780572	transmembrane, SP not likely real	6	1	1. Function, pilus transmembrane component; 2. single-TMH, major part periplasmic
254780679	transmembrane, SP not likely real	6	2	might be single transmembrane protein, and the C-terminal is cytoplasmic
254780671	transmembrane, SP not likely real	6	2	1. function; 2. might be single-TMH after cleaving SP, major part is cytoplasmic
254780532	transmembrane, SP not likely real	5	2	1. single-TMH, major part periplasmic; 2. ortholog can be proved by only one mode of signalp
peg_237	transmembrane, SP not likely real	6	2	single-TMH in the middle
peg_789	transmembrane, SP not likely real	6	2	multi-TMHs
254781098	transmembrane, SP not likely real	6	2	Multi-TMHs
254780896	transmembrane, but SP is probably real	6	2	1. function; 2. multi-TMHs
254780372	transmembrane, but SP is probably real	6	3	1. function, flagellar transmembrane component; 2. Multi-TMHs
254780729	transmembrane, but SP might be true	6	3	1. function; 2. Multi-TMHs
254780196	transmembrane, but SP might be true	6	3	1. function; 2. after cleavage of SP, single-TMH, major cytoplasmic
254780468	transmembrane, but SP is probably real	6	4	Multi-TMHs

N1: number of methods (out of 6) that predict the protein to have transmembrane helix (helices)

N2: number of methods (out of 4) that predict the protein to have a signal peptide

SP: signal peptide

TMH: transmembrane helix

Cell color:

Yellow: extracytoplasmic proteins that are without signal peptides and that are secreted in Sec-independent pathways;

Light green: proteins with signal peptides and they are likely function in the extracytoplasmic space;

Pink: cytoplasmic proteins

Light blue: transmembrane proteins that are located in the inner membrane of this Gram-negative bacterium.