

	Walker-A motifs	Walker-B motifs
VirB11 homologues		
<i>Helicobacter pylori</i> WP_000133885	NVIVC CGTGSGK [*] TTYIK	LRMRPD RIILGE [*] LRSS
<i>Brucella suis</i> AAN33271	VIVVAG ETGSGK TTLMK	LRMKPT RILLAE LRGGE
<i>Sinorhizobium meliloti</i> AAK65367	NIIIS GATGSGK TTLISK	LRMRPD RILLQE LRDGT
<i>Xylella fastidiosa</i> WP_010895212	TLVIV GKTGSGK TTIGK	LRMKPD RILLTE MRGDE
<i>Agrobacterium fabrum</i> NP_396558	NIVIS GGTGSGK TTLAN	MRLRPD RIIVGE VRDGA
<i>Pseudomonas aeruginosa</i> NP_252992	NILIS GGTGTGK TTLN	LRMRPD RIILGE IRGVE
<i>Ralstonia solanacearum</i> WP_011002499	NILIA GATSSGK TTLAN	MRLRPD RVIVGE VRGGE
VirD4 homologues		
<i>Helicobacter pylori</i> WP_000389328	FIGLIA PTRSGK GVGFI	QCKRSC LMLMDE [*] FTLCG
<i>Rickettsia prowazekii</i> NP_220677	HALLF APTGSGK GVGFV	-EPYGV MFLLE FPFLG
<i>Agrobacterium fabrum</i> NP_396505	HSLVV APTRAGK GVGVV	--RHEV LFLLE EFKHLG
<i>Xylella fastidiosa</i> WP_010895213	HVSIS APTRSGK GVGIV	ALKYQC LLMMDE FTALG
<i>Agrobacterium fabrum</i> NP_355804	HLLTM APTRT GKGVGTI	--ATPV LYLLE EFASLG
<i>Mesorhizobium japonicum</i> WP_010915832	HVLVV GPTRSGK GVSVFV	--PYQV LVMI DEFRLG
<i>Clostridium acetobutylicum</i> NP_348589	NIAVF GASGSMK SRSFV	EAQKEV YFILDE FPNIG
<i>Sinorhizobium meliloti</i> NP_435748	HMLFF AGSGGYK TTSNV	-FKRRAL LFMLDE VDLLG
<i>Agrobacterium fabrum</i> NP_396647	HGIVF AGSGGFK TTSVT	-VKGRT LFLLE AVARLG
VirB4 homologues		
<i>Helicobacter pylori</i> WP_000495985	HTLII LGSTGSGK TVFMS	DGRRF- VLDIDE [*] AWKYL
<i>Escherichia coli</i> AAA86452	HALIT GMSGEGK TTLN	DGKRRV- IQCFDE FHAYL
<i>Escherichia coli</i> CAA57024	NTMLI GQSSSGK TVLLG	DGRRF- MYVFDE FWKPL
<i>Bartonella henselae</i> AAF00942	NTFVC GPSGSGK TVIVN	DGRRF- IIVIDE FWKAL
<i>Agrobacterium tumefaciens</i> CAA37357	MTAIF GPPIGRGK TTLMT	DGRRF- VMSCDE FRAYL
<i>Legionella pneumophila</i> CAB60053	HAAIF GGNNAGK TTLVN	DGRLT- SFI IAE AWQLF
<i>Novosphingobium aromaticivorans</i> AAD03958	NVAVF GKSGSGK SVFLQ	DRSVPK LTMI DEAWQLL
<i>Rickettsia prowazekii</i> WP_004599743	HTLII GPTGAGK TVLMN	DGQKT- MIVLDE AWALI

Supplemental Figure 1: Identification of Walker boxes in Cag T4SS ATPases. The sequences of *H. pylori* Cag T4SS ATPases were aligned with sequences of the most closely related T4SS ATPases in other bacterial species. Genbank accession numbers are listed for each sequence. Blue boxes and red boxes indicate Walker-A motifs and Walker-B motifs, respectively. Asterisks indicate the amino acids targeted for mutagenesis.