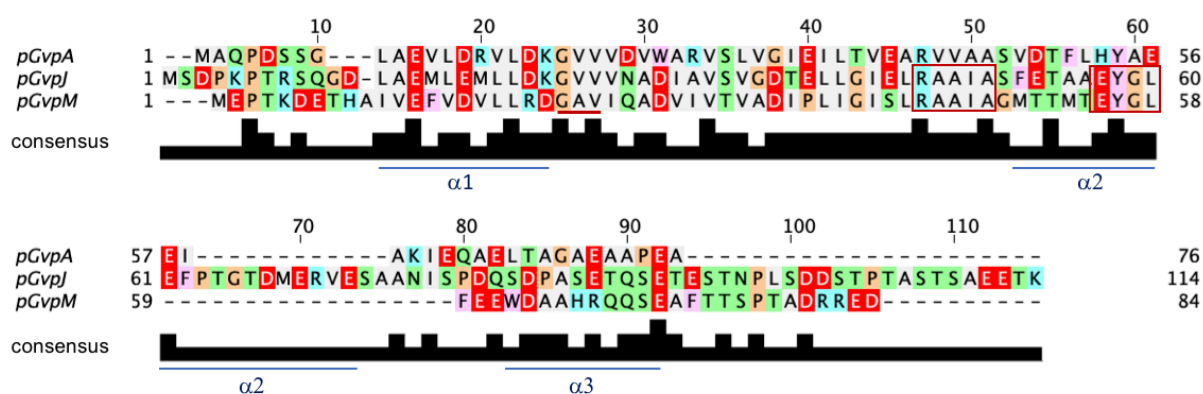


Supplementary Figures and Tables

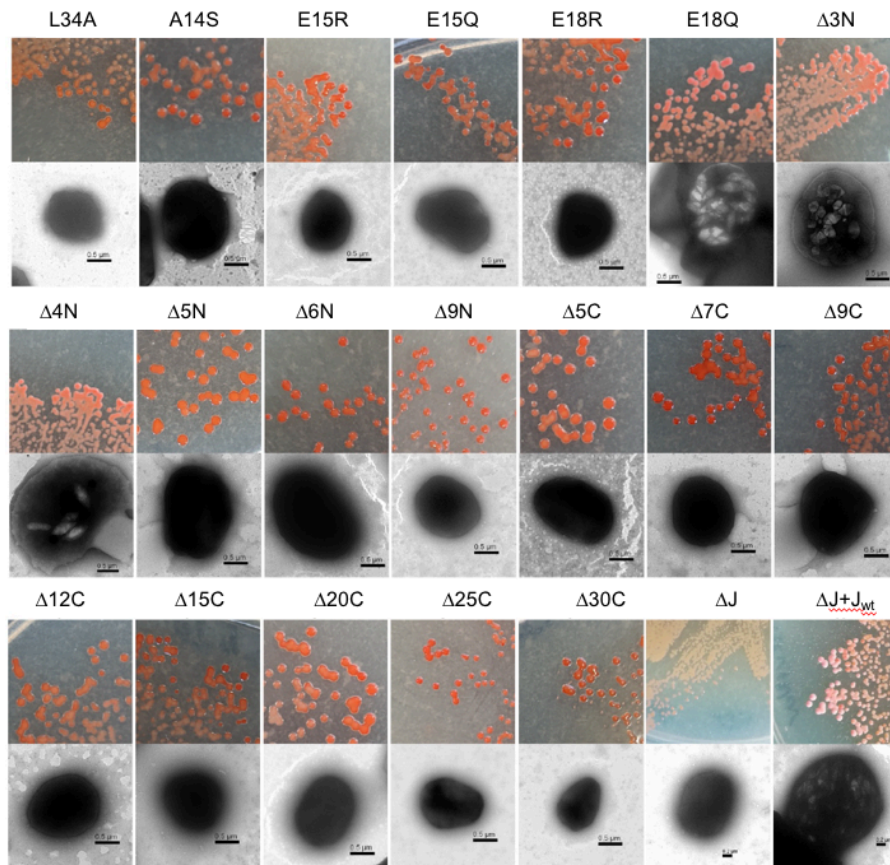
Effect of Mutations in GvpJ and GvpM on Gas Vesicle Formation of *Halobacterium salinarum*

Alisa Jost¹, Regine Knitsch¹, Kerstin Völkner¹, and Felicitas Pfeifer^{1*}

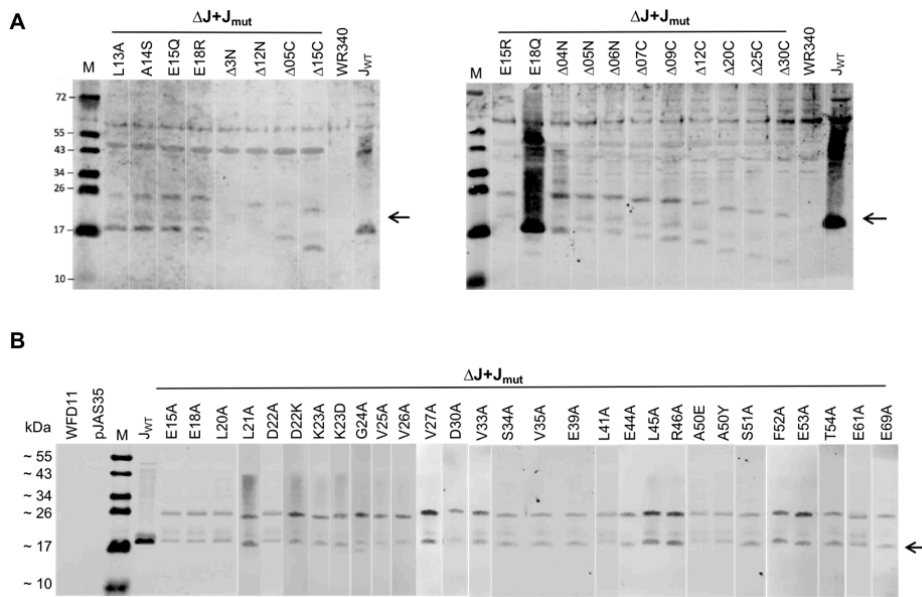
¹Microbiology and Archaea, Department of Biology, Technical University Darmstadt, Darmstadt, Germany



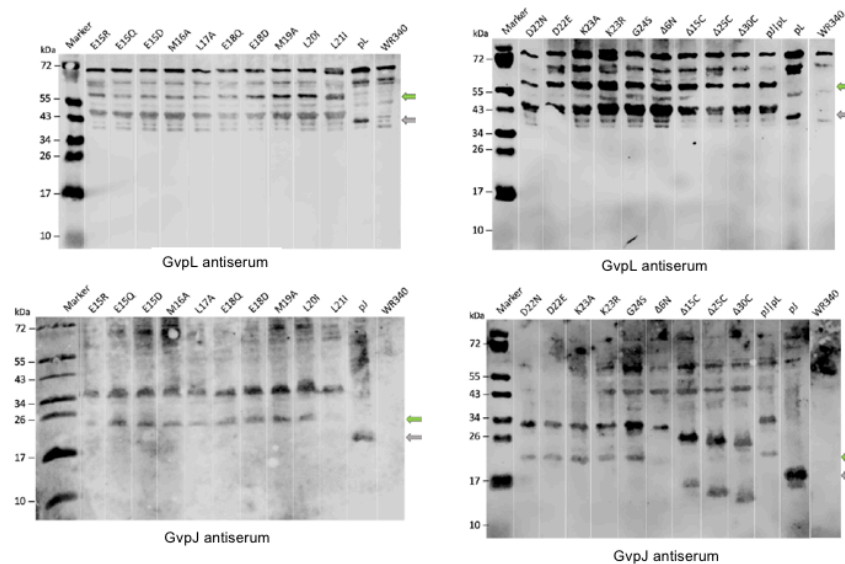
Supplementary Figure S1. Alignment of the aa sequence of GvpA, GvpJ and GvpM derived from the p-vac region of *Halobacterium salinarum* PHH1. The highlighted sequence motifs of GvpJ and GvpM (GAV, RAAIA, EYGL) are discussed in the text. The consensus of the three sequences depicted by black beams is shown below. Predicted alpha-helices of GvpJ are labelled alpha1 through alpha3.



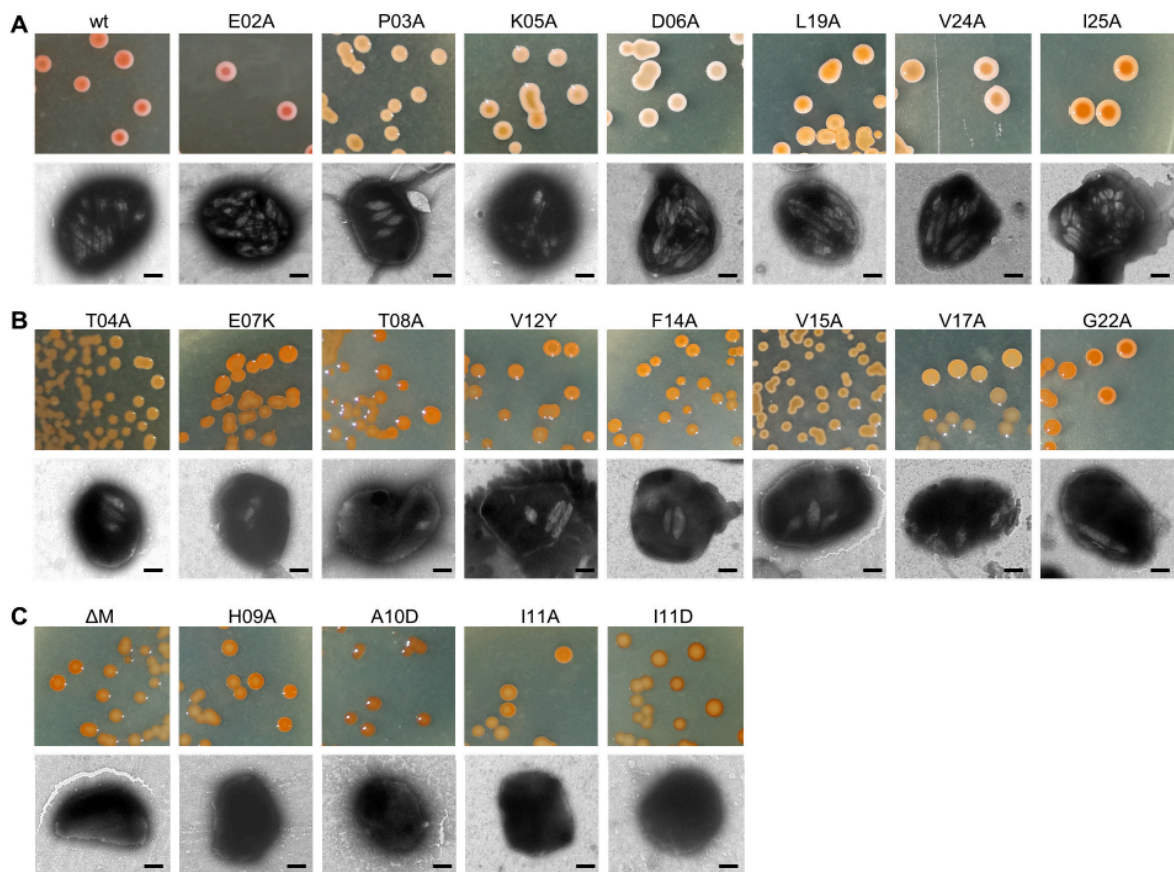
Supplementary Figure S2. Colonies of $\Delta J+J_{wt}$ and $\Delta J+J_{mut}$ transformants on solid media (top), and cells analyzed by transmission electron microscopy (underneath). The respective GvpJ variant is labelled on top. Colonies of gas vesicle containing cells are turbid and pink (see $\Delta J+J_{wt}$ bottom row, right). The red color is due to the carotene bacterioruberin. In contrast, colonies of Vac^- cells are transparent. The difference in the darkness of the red color depends on the oxygen supply during growth. Cells of several colonies were inspected by transmission electron microscopy for the possession of gas vesicles. In each case, a representative cell is shown underneath.



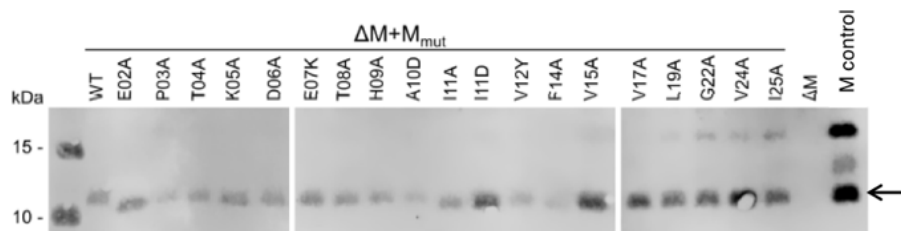
Supplementary Figure S3. Western analyses to determine the presence of GvpJ. Total proteins were isolated from $\Delta J+J_{wt}$ or $\Delta J+J_{mut}$ transformants, and 20 μg of proteins were separated by SDS-PAGE, followed by transfer on a PVDF membrane and treatment with an antiserum raised against GvpJ. The respective substitution- or deletion variant is indicated on top. Arrows on the right mark the position of GvpJ.



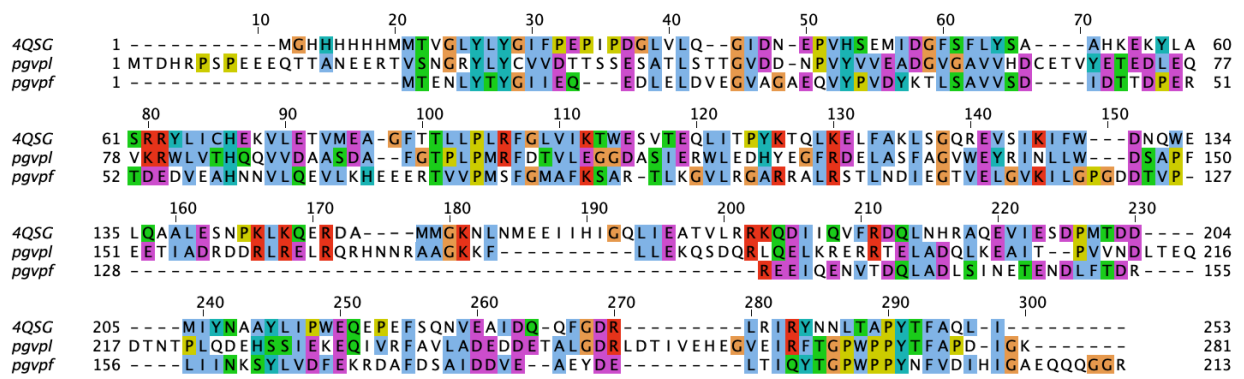
Supplementary Figure S4. Western analysis to determine the presence of GvpJ and GvpL in the interaction study. Total proteins were isolated from cells containing J_{mut} -CGFP and NGFP-L, and 20 μg of proteins were separated by SDS-PAGE, followed by transfer to a PVDF membrane and treatment with the respective antiserum to detect NGFP-L (top) or JCGFP (bottom). Lysates of *Hfx. volcanii* transformants expressing *gvpJ*-pJAS35 or *gvpL*-pJAS35 were used as control. Green arrows mark the position of the expected Gvp-GFP fusion protein, grey arrows the original GvpJ or GvpL.



Supplementary Figure S5. Colonies of $\Delta M+M_{wt}$ and $\Delta M+M_{mut}$ transformants on solid media (top), and representative cells analyzed by transmission electron microscopy for the possession of gas vesicles. The respective GvpJ variants are labeled on top. **(A)** Transformants producing gas vesicles similar to wild type (wt) (Vac^+ phenotype). **(B)** Transformants containing a few gas vesicles only (Vac^\pm). **(C)** Transformants exhibiting a Vac^- phenotype. Further explanations are given in the text.



Supplementary Figure S6. Western analyses to determine the presence of GvpM. Total proteins were isolated from $\Delta M+M_{wt}$ or $\Delta M+M_{mut}$ transformants, and 20 μg of proteins were separated by SDS-PAGE followed by transfer on a PVDF membrane and treatment with an antiserum raised against GvpM. The respective substitution of deletion variant is indicated on top. The arrow on the right marks the position of GvpJ.



Supplementary Figure S7. Alignment of GvpF derived from the cyanobacterium *Microcystis aeruginosa* and the GvpF and GvpL proteins of the haloarchaeon *Halobacterium salinarum*.

Supplementary Table 1. Oligonucleotides used in this study.

Name **Oligonucleotide sequence (5'-3')***

p-*gvpM* substitutions in M^{Ex}

f-pM/E02A	atgg <u>ca</u> ccaacaaaagacgag
f-pM/P03A	atggaggcaacaaaagacgag
f-pM/T04A	atggagccagcaaaaagacgag
f-pM/K05A	atggagccaacag <u>c</u> agacgag
f-pM/D06A	atggagccaacaaaag <u>cc</u> gag
f-pM/E07K	gac <u>a</u> gacacacgcgatcgtt
f-pM/T08A	gacgaggcacacgcgatcgtt
f-pM/H09A	gacgagacag <u>cc</u> gcgatcgtt
f-pM/A10D	gacgagacacacg <u>a</u> catcgtt
f-pM/I11A	g <u>cg</u> g <u>cc</u> ggttgagttcgt
f-pM/I11D	g <u>cg</u> g <u>a</u> cggttgagttcgt
f-pM/V12Y	gcgatc <u>t</u> atgagttcgt
f-pM/F14A	gcgatcgttgagg <u>cc</u> gt
f-pM/V15A	ttcg <u>cc</u> gacgtgttactgc
f-pM/V17A	ttcgtcgacg <u>c</u> gttactgc
f-pM/L18A	ttcgtcgacgtg <u>gc</u> actgc
f-pM/L19A	ttcgtcgacgtgttag <u>cg</u> c
f-pM/G22A	gacg <u>c</u> agccgtgattcaag
f-pM/V24A	gacggagccg <u>c</u> gattcaag
f-pM/I25A	gacggagccgtg <u>gc</u> tcaag
r-pM/E02, P03, T04, K05, D06	ctgcagagttcggcttcc
r-pM/E07, T08, H09, A10	ttttgttgctccatctgcagag
r-pM/I11, V12, F14	gtgtgtctcgtctttgttgctc
r-pM/V15, V17, L18, L19	ctcaacgatcgcgtgtgtctc
r-pM/G22, V24, I25	gcgcaagtaacacgtcgac
f-pM/D28G	aagcggg <u>cg</u> tgatcgt
r-pM/D28G	gaatcacggctccgtc
f-pM/T32K	gtgatcgtga <u>gg</u> tcgccg
r-pM/T32K	gtccgcttgaatcacggctc

p-*gvpM* substitutions in split-GFP vector pM(25N)_N

pM_25N-E02A_fwd	atgcccatgg <u>cg</u> ccaacaaa
pM_25N-E02A_rev	cactgcagagttcggcttccga
pM_25N-P03A_fwd	atgcccatggaggcaacaaa
pM_25N-T04A_fwd	atgcccatggagccagcaaaaag
pM_25N-K05A_fwd	gccaacag <u>c</u> agacgagacac
pM_25N-K05A_rev	tccatgggcatcactgcagag
pM_25N-D06A_fwd	gccaacaaaag <u>ca</u> gagacacacg
pM_25N-E07A_fwd	gccaacaaaagacg <u>cg</u> acacac
pM_25N-T08A_fwd	gccaacaaaagacgaggcacacg
pM_25N-H09A_fwd	cgagacag <u>cc</u> gcgatc
pM_25N-H09A_rev	tctttgttgctccatgggcatc
pM_25N-A10D_fwd	cgagacacac <u>tg</u> gatcgttgag
pM_25N_I11A_fwd	acacg <u>cg</u> g <u>ca</u> ggttgagttc
pM_25N_I11D_fwd	acacg <u>cg</u> g <u>a</u> cggttgagttc
pM_25N_V12Y_fwd	acacgcatc <u>t</u> atgagttcgtcg
pM_25N_E13A_fwd	acacgcatcgtt <u>gc</u> atcgtc
pM_25N_I11A_rev	gtctcgtctttgttgctccatg

3'-BspI-pM(25N)_D21A	tgttgt gctcage cc aatcacggctccggc
3'-BspI-pM(25N)_G22A	tgttgt gctcage cc aatcacggctgcgtc
3'-BspI-pM(25N)_A23D	tgttgt gctcage cc aatcacgtctccgctgc
3'-BspI-pM(25N)_V24A	tgttgt gctcage cc aatcgcggctccgctc
3'-BspI-pM(25N)_I25A	tgttgt gctcage cc agccacggctccg
5'-NcoI-pM	tgttgt ccatgg atggagccaacaaaag
3'-BspI-pM(25N) Δ stop	tgttgt gctcage cc aatcacggctccgctc

p-gvpM substitutions in split-GFP vector pM_C

5'-NcoI-pM	tgttgt ccatgg atggagccaacaaaag
3'-BamHI-pM Δ stop	agttct ggatcc c gtcctctcgcgc

p-gvpJ substitutions in pBSK(II)+ and pJAS35

5'-PstI-pJ	attc ctgcag atg agtgaccccaaacgcac
3'-KpnI-pJ	attc ggatcc tea tttggtctctccgctg
f-pJ/L13A	gcgac gcag ccgagatg
f-pJ/A14S	gcgacctc ccg agatg
r-pJ/L13, A14	cctgcgagc gcg ctc
f-pJ/L13I	<u>a</u> tcg ccg agatgctggagatgc
f-pJ/A14V	ctc gcg agatgctggagatgctc
f-pJ/A14D	ctc gcg agatgctggagatgctc
r-pJ/L13, A14	gtcgc ccctgcgagcgcg
f-pJ/E15A	ggcgacctc gccg gatgctggagatg
r-pJ/E15A	catctccagcatc gcggc gaggtcgcc
f-pJ/E15R	ctc gccagg atgctggag
f-pJ/E15Q	ctc gcc agatgctggag
f-pJ/E15D	ctc gccg atgctggagatg
r-pJ/E15	gtcgc ccctgcgagc
f-pJ/M16A	gag gcg ctggagatgc
f-pJ/L17A	gagat ggc ggagatgctcc
f-pJ/E18R	gagatgct gc agatgctccta
f-pJ/E18Q	gagatgct gagg atgctccta
f-pJ/E18D	gagatgct gga tatgctcctagac
r-pJ/M16, L17, E18	ggcgaggtc gccc
f-pJ/E18A	gccgagatgct ggc gatgctcctagac
r-pJ/E18A	gtctaggagcat gcc agcatctcgg
f-pJ/E18M	<u>a</u> tgatgctcctagacaaaggagtcgctc
f-pJ/M19E	gag ga gctcctagacaaaggagtcgctc
f-pJ/E18, M19	cagcatctc ggc gaggtcgc
f-pJ/M19A	gag gcg ctcctagacaaagg
f-pJ/L20A	atgctggagat ggc gctagacaaagga
r-pJ/L20A	tcctttgtctag cc catctccagcat
f-pJ/L20I	gagatg at cctagacaaaggagtcgctc
f-pJ/L21I	gagatgctc at agacaaaggagtcgctc
f-pJ/D22N	gagatgctcct aa agagtcg
f-pJ/D22E	gagatgctcctag aa agagtcgctc
r-pJ/M19, L20, L21, D22	cagcatctc ggc gaggtc
f-pJ/L21A	<u>gc</u> agacaaaggagtcgctgctcaac
f-pJ/D22K	ct aa agagtcgctgctcaacgc
f-pJ/K23D	ctagac gaf ggagtcgctgctcaacg
f-pJ/D22A	gagatgctcctag g aaagagtcgctc
r-pJ/D22A	gacgactcctt cg ctaggagcatctc

r-pJ/L21, K23
f-pJ/K23A
f-pJ/K23R
f-pJ/G24S
r-pJ/K23, G24
f-pJ/G24A
f-pJ/V25A
f-pJ/V26A
f-pJ/V27A
r-pJ/G24, V25, V26, V27
f-pJ/A29D
f-pJ/D30A
r-pJ/A29, D30
f-pJ/V33A
f-pJ/V33D
r-pJ/V33
f-pJ/S34A
f-pJ/V35A
r-pJ/S34, V35
f-pJ/E39A
f-pJ/L41A
r-pJ/E39, L41
f-pJ/E44A
f-pJ/L45A
f-pJ/R46A
r-pJ/E44, L45, R46
f-pJ/R46E
f-pJ/A47D
f-pJ/A48D
r-pJ/R46, A47, A48
f-pJ/I49A
f-pJ/A50D
r-pJ/I49, A50
f-pJ/A50Y
f-pJ/A50E
f-pJ/S51A
r-pJ/A50, S51
f-pJ/F52A
f-pJ/E53A
f-pJ/T54R
r-pJ/F52, E53, T54
f-pJ/T54A
r-pJ/T54A
f-pJ/A55D
f-pJ/A56D
r-pJ/A55, A56
f-pJ/Y58A
r-pJ/Y58A
f-pJ/E57A
f-pJ/Y58E
f-pJ/G59A
r-pJ/E57, Y58, G59
f-pJ/L60A
r-pJ/L60A
f-pJ/E61A
r-pJ/E61A

gagcatctccagcatctcggc
gacgcaggagtcgtcgtc
gac aga gga gtc gtc gtc aac
gac aaa agt gtc gtc gtc aac g
taggagcatctccagcatctcg
gcagtcgtcgtcaacgcggatatcg
ggagcagtcgtcaacgcggatatcg
ggagtcgcagtcacgcggatatcg
ggagtcgtcgcaaacgcggatatcg
ttgtctaggagcatctccagcatctcgg
gacgatatcgagtcagcgtcgg
gcggctatcgagtcagcgtc
gttgacgacgactcctttgtctaggagc
gccagcgtcggggacacgg
gacagcgtcggggacacggaac
tgcgatatccgcgttgacgacg
gcagtcggggacacggaactcctc
agcgcagggggacacggaactcc
gactgcgatatccgcgttgacgacg
gcactcctcggtatcgagttacgggc
gaactgcaggtatcgagttacgggcc
cgtgtccccgacgctgactgc
gcattacggggccgcgattgcttc
gaggcacggggccgcgattgc
gagttagcagccgcgattgcttcgttc
gataccgaggagttccgtgtccccgac
gagggccgcgattgcttcgttc
cgggacgcgattgcttcgttc
cgggccgacattgcttcgttc
taactcgataccgaggagttccgtgtc
gctgcttcgttcgagacagcggc
attgattcgttcgagacagcggcc
cgccggcccgttaactcgataccg
tattcgttcgagacagcggccgaatac
gaatcgttcgagacagcggccgaatac
gctgcattcgagacagcggccg
aatcgccggcccgttaactcgatacc
gccgagacagcggccgaatac
ttcgcagacagcggccgaatac
ttcgagagagcggccgaatac
cgaagcaatcgccggccc
gcagcggccgaatacgggctc
ctcgaacgaagcaatcgccggcc
gacgcccgaatacgggctcgag
gcggacgaatacgggctcgag
tgtctgaacgaagcaatcgcg
gcagggctcgagttccaacgg
ttcgccgctgtctcgaacgaag
gcttacgggctcgagttccaac
gaagaggggctcgagttccaac
gaatacgcctcgagttccaacgg
ggccgctgtctcgaacgaagc
gctgagttccaacggggacg
cccgtattcgccgctgtctc
gcattccaacggggacggatatg
gagcccgtattcgccgctgtc

f-pJ/E69A	<i>gcacgcgctcagtcgccc</i>
r-pJ/E69A	<i>catatccgtccccgttgggaactcg</i>
f-pJ/A74D	<i>gcacgcgaatatctcaccggaccag</i>
r-pJ/A74D	<i>ggactcgacgcgctccatatec</i>

p-gvpJ deletions in pBSK(II)+ and pJAS35

f-Start+PstI-pJxpBSK+	<i>catctgcagccccggg</i>
r-Δ3N-pJ	<i>cccaaaccgacgcgc</i>
r-Δ4N-pJ	<i>aaaccgacgcgctcg</i>
r-Δ5N-pJ	<i>ccgacgcgctcg</i>
r-Δ6N-pJ	<i>acgcgctcgcagg</i>
r-Δ9N-pJ	<i>cagggcgacctcg</i>
r-Δ12N-pJ	<i>ctcgcggagatgctgg</i>
f-Δ5C-pJ	<i>tgacgtggatgccgtc</i>
f-Δ7C-pJ	<i>ggatgccgtcggcg</i>
f-Δ9C-pJ	<i>cgtcggcgtcgaatcgtc</i>
f-Δ10C-pJ	<i>cggcgtcgaatcgtcc</i>
f-Δ12C-pJ	<i>cgaatcgtccgacagtggg</i>
f-Δ15C-pJ	<i>cgacagtgggtcgtcgac</i>
f-Δ20C-pJ	<i>cgactcggctcggattgg</i>
f-Δ25C-pJ	<i>ttgggtttccgacgccg</i>
f-Δ30C-pJ	<i>cgggtcggactggtcc</i>
r-Stop+KpnI-pJxpBSK+	<i>tgaggtacccaattcgcct</i>
5'-PstI-pJ	<i>attc ctgcag atg agtgaccccaaacccgac</i>
3'-KpnI-pJ	<i>attc ggtaac tca tttggtctctcctcgctg</i>
5'-PstI-pJΔ3N	<i>attc ctgcag atg cccaaaccgacgc</i>
5'-PstI-pJΔ4N	<i>attc ctgcag atg aaa ccg acg cgc tcg</i>
5'-PstI-pJΔ5N	<i>attc ctgcag atg ccg acg cgc tcg</i>
5'-PstI-pJΔ6N	<i>attc ctgcag atg cccaaaccgacgc</i>
5'-PstI-pJΔ9N	<i>attc ctgcag atg cagggcgacctc</i>
5'-PstI-pJΔ12N	<i>attc ctgcag atg ctcgcggagatgc</i>
3'-KpnI-pJΔ5C	<i>attc ggtaac tca tgacgtggatgccgtc</i>
3'-KpnI-pJΔ7C	<i>attc ggtaac tca ggatgccgtcggcg</i>
3'-KpnI-pJΔ9C	<i>attc ggtaac tca cgtcggcgtcgaatcgtc</i>
3'-KpnI-pJΔ10C	<i>attc ggtaac tca cggcgtcgaatcgtc</i>
3'-KpnI-pJΔ12C	<i>attc ggtaac tca cgaatcgtccgacagtggg</i>
3'-KpnI-pJΔ15C	<i>attc ggtaac tca cgacagtgggtcgtc</i>
3'-KpnI-pJΔ20C	<i>attc ggtaac tca cgactcggctcgcg</i>
3'-KpnI-pJΔ25C	<i>attc ggtaac tca ttgggtttccgacgc</i>
3'-KpnI-pJΔ30C	<i>attc ggtaac tca cgggtcggactgg</i>

Split-GFP plasmids gvpJ (substitutions, fragments, deletions)

5'-BspHI-pJ	<i>actgac tcata gtgaccccaaacccg</i>
3'-BamHI-pJΔStop	<i>tgatt ggatcc c tttggtctctcctcgc</i>
5'-BamHI-pJ	<i>aggtcaa ggatcc atgagtgaccccaaac</i>
3'-BspI-pJ_1-56 +Stop	<i>attc gctcagc tca ggccgctgtctcgaac</i>
3'-BspI-pJ_1-56	<i>attc gctcagc ga ggccgctgtctcgaac</i>
3'-BamHI-pJ_1-56	<i>attc ggatcc c ggccgctgtctcgaac</i>
3'-KpnI-pJ +Stop	<i>attc ggtaac tca ggccgctgtctcgaac</i>
5'-NcoI-pJ_47-114	<i>attc ccatgg ccgcgattgcttcg</i>
5'-BamHI-pJ_47-114	<i>attc ggatcc atg gccgcgattgcttcg</i>
3'-BspI-pJ	<i>gtaacct gctcagc tcatttggctctcctcg</i>

3'-BspI-pJΔStop	aagtaacct gctcage cctttggtctctcegc
3'-KpnI-pJ	attc ggtacc tcatttggtctctcegcgtg
5'-NcoI-pJΔ3N	attc ccatgg ca atg cccaaaccgacgc
5'-BspHI-pJΔ4N	attc tcatga aaccgacgcgctcg
5'-NcoI-pJΔ5N	attc ccatgg ca atg cgcgacgcgctcg
5'-BspHI-pJΔ6N	attc tcatga cgcgctcgag
5'-NcoI-pJΔ12N	attc ccatgg ca atg ctcgccgagatgc
3'-BamHI-pJΔ5C	attc ggatcc c tgacgtggatgccgtc
3'-BamHI-pJΔ7C	attc ggatcc c gcatgccgtcggcg
3'-BamHI-pJΔ9C	attc ggatcc c cgtcggcgtcgaatcgtc
3'-BamHI-pJΔ10C	attc ggatcc c cggcgtcgaatcgtc
3'-BamHI-pJΔ12C	attc ggatcc c cgaatcgtcgcacagtggg
3'-BamHI-pJΔ15C	attc ggatcc c cgacagtgggttcgtc
3'-BamHI-pJΔ20C	attc ggatcc c cgactcggctcgcg
3'-BamHI-pJΔ25C	attc ggatcc c ttgggtttccgacgc
3'-BamHI-pJΔ30C	attc ggatcc c cgggtcggactgg

*restriction sites are marked in bold; codons for substituted aa are marked in *italics* and are underlined; nucleotides added are separated by blanks

Supplementary Table S2. Effect of mutations in GvpJ on gas vesicle formation

Substitution	position	Vac/GV*	J/L rf value*
L13A	α 1	negative	---
L13I	α 1	few	---
A14D	α 1	negative	---
A14V	α 1	negative	---
E15A/D/R	α 1	negative	---
E15Q	α 1	negative	higher
M16A	α 1	negative	---
L17A	α 1	negative	---
E18A/M/D/R	α 1	negative	---
E18Q	α 1	wt	---
M19A	α 1	negative	---
M19E	α 1	negative	lower
L20A	α 1	negative	lower
L20I	α 1	negative	---
L21A/I	α 1	negative	---
D22A	α 1	negative	---
D22E	α 1	negative	higher
D22N	α 1	negative	---
D22K	α 1	spindle/cylinder	--
K23A	loop1	cylinder/ unstable	---
K23D	loop1	negative	---
G24A	loop1	negative	lower
G24S	loop1	negative	lower
V25A	β 1	spindle/ unstable	---
V26A	β 1	negative	---
V27A	β 1	negative	---
A29D	β 1	negative	higher
D30A	β 1	negative	---
V33A/D	β 1	negative	higher
S34A	β 1	wt	---
V35A	β 1	negative	---
E39A	loop 2	few	---
L41A	β 2	negative	---
E44A	β 2	negative	---
L45A	β 2	spindle	---
R46A	β 2	cylinder/ unstable	---
R46E	β 2	negative	---
A47D	β 2	negative	higher
A48D	β 2	negative	higher
I49A	β 2	negative	higher
A50D/E	β 2	negative	higher
A50Y	β 2	negative	---
S51A	β 2	negative	higher
F52A	α 2	negative	higher
E53A	α 2	negative	---
T54A/R	α 2	negative	---
A55D	α 2	negative	---
A56D	α 2	negative	---
E57A	α 2	negative	---

Y58A	$\alpha 2$	negative	---
Y58E	$\alpha 2$	negative	lower
G59A	$\alpha 2$	negative	---
L60A	$\alpha 2$	negative	higher
E61A	$\alpha 2$	negative	---
E69A	$\alpha 2$	cylinder	---
A74D	loop3	negative	---
J _{WT}		wt	---

 *Vac phenotype: wt, wild type; few = few gas vesicles; Rf --- = similar to wild type
 GV, gas vesicle shape

Supplementary Table S4. Split-GFP analyses investigating interactions of A-J-M fragments. The fluorescence was measured in LAU/mm² and the relative fluorescence calculated.

transformant		LAU/mm ²	σ LAU/mm ²	Relative fluorescence (rf)	transformant		LAU/mm ²	σ LAU/mm ²	Relative fluorescence (rf)
control									
	WR340	17,066	1,687	0.00					
GvpJ interactions									
					NJ1-56	A44-76c	18,063	1,269	0.00
						cA44-76	14,600	861	0.00
					J1-56N	A44-76c	15,315	337	0.00
NJ	A1-22c	15,515	1,185	0.06		cA44-76	13,922	1,686	0.00
	cA1-22	13,310	1,117	0.00	cJ1-56	A44-76N	16,683	1,749	0.00
JN	A1-22c	13,310	1,117	0.00		N A44-76	16,268	432	0.00
	cA1-22	12,931	1,974	0.01	J1-56c	A44-76N	18,383	534	0.00
cJ	A1-22N	15,603	779	0.05		N A44-76	18,091	508	0.00
	N A1-22	17,016	637	0.14					
Jc	A1-22N	25,287	2,336	0.70					
	N A1-22	40,430	2,947	1.71	NJ1-56	Mc ¹	105,270	3,549	0.34
						cM ¹	111,793	11,823	0.42
					J1-56N	Mc ¹	84,466	7,502	0.08
NJ	A44-76c	11,772	569	0.10		cM ¹	118,065	18,329	0.35
	cA44-76	10,993	893	0.05	cJ1-56	MN ¹	111,356	7,800	0.27
JN	A44-76c	10,841	867	0.04		NM ¹	119,482	6,313	0.37
	cA44-76	11,023	1,328	0.07	J1-56c	MN ¹	100,725	3,806	0.15
cJ	A44-76N	11,040	1,271	0.06		NM ¹	95,150	2,439	0.09
	N A44-76	9,293	2,029	0.01					
Jc	A44-76N	21,842	1,550	1.03					
	N A44-76	16,121	1,185	0.50	NJ1-56	M1-25c	291,164	4,975	18.05
						cM1-25	298,948	10,258	18.56
					J1-56N	M1-25c	276,530	22,476	17.09
NJ	M1-25c	16,685	1,969	0.66		cM1-25	275,376	15,054	17.02
	cM1-25	11,355	1,839	0.14	cJ1-56	M1-25N	273,638	10,757	16.90
JN	M1-25c	16,946	951	0.68		NM1-25	250,688	5,141	15.40
	cM1-25	12,195	1,030	0.21	J1-56c	M1-25N	286,298	7,624	17.73
cJ	M1-25N	11,296	1,514	0.15		NM1-25	284,322	11,170	17.60
	NM1-25	10,935	1,523	0.10					
Jc	M1-25N	15,022	963	0.49					
	NM1-25	15,789	1,493	0.57	NJ1-56	M60-84c	306,437	6,388	20.75
						cM60-84	280,733	7,194	18.93
					J1-56N	M60-84c	289,429	4,594	19.55
NJ	M60-84c	16,396	458	0.01		cM60-84	301,170	8,442	20.38
	cM60-84	15,530	622	0.00	cJ1-56	M60-84N	283,697	6,585	19.14
JN	M60-84c	17,799	834	0.08		NM60-84	289,387	7,955	19.54
	cM60-84	16,423	1,561	0.03	J1-56c	M60-84N	309,739	9,921	20.99
cJ	M60-84N	15,259	887	0.00		NM60-84	281,190	11,376	18.96
	NM60-84	17,011	1,595	0.05					
Jc	M60-84N	20,220	2,125	0.23					
	NM60-84	47,920	3,298	1.91	NJ47-114	Ac	34,030	3,212	0.14
						cA	17,794	886	0.00
					J47-114N	Ac	112,572	93,078	2.93
						cA	18,683	205	0.00
GvpJ fragments with GvpA/GvpM									
					cJ47-114	AN	36,273	19,382	0.69
NJ1-56	Ac	19,055	726	0.00		NA	19,039	709	0.00
	cA	14,995	1,428	0.00	J47-114c	AN	17,438	353	0.00
J1-56N	Ac	15,844	369	0.00		NA	18,226	942	0.00
	cA	15,260	750	0.00					
cJ1-56	AN	15,867	1,088	0.00					
	NA	12,791	455	0.00	NJ47-114	A1-22c	27,128	310	0.35
J1-56c	AN	14,375	1,302	0.00		c A1-22	12,503	669	0.00
	NA	14,825	463	0.00	J47-114N	A1-22c	14,619	613	0.00
						c A1-22	12,024	1,194	0.00
					cJ47-114	A1-22N	154,695	136,769	5.71
NJ1-56	A1-22c	14,821	859	0.00		N A1-22	20,322	1,074	0.00
	cA1-22	12,163	192	0.00	J47-114c	A1-22N	11,812	812	0.00
J1-56N	A1-22c	13,865	559	0.00		N A1-22	16,591	2,831	0.02
	cA1-22	12,505	252	0.00					
cJ1-56	A1-22N	243,810	6,391	9.06					
	N A1-22	129,406	114,911	4.54					
J1-56c	A1-22N	243,821	9,884	9.06					
	N A1-22	19,084	1,041	0.00					

¹ = LAU value of *Hfx. volcanii* WR340: 87,354

continued

transformant		LAU/mm ²	σ LAU/mm ²	Relative fluorescence (rf)	transformant		LAU/mm ²	σ LAU/mm ²	Relative fluorescence (rf)
NJ47-114	A44-76 _C	26,216	1,771	0.31	NJ47-114	H _C	272,516	6,881	12.36
	cA44-76	11,494	723	0.00		cH	20,393	775	0.02
J47-114 _N	A44-76 _C	14,541	320	0.00	J47-114 _N	H _C	29,273	1,266	0.44
	cA44-76	12,025	728	0.00		cH	14,794	827	0.00
cJ47-114	A44-76 _N	149,838	132,004	5.51	cJ47-114	H _N	15,861	844	0.00
	nA44-76	19,857	305	0.00		nH	15,230	975	0.00
J47-114 _C	A44-76 _N	12,264	1,280	0.00	J47-114 _C	H _N	14,412	850	0.00
	nA44-76	13,501	364	0.00		nH	14,023	1,437	0.00
NJ47-114	M _C	35,279	3,507	0.70	NJ47-114	l _C	13,982	1,228	0.00
	cM	14,559	168	0.00		cl	24,422	457	0.12
J47-114 _N	M _C	16,775	219	0.00	J47-114 _N	l _C	15,227	1,739	0.00
	cM	13,815	1,401	0.00		cl	17,491	1,015	0.00
cJ47-114	M _N	12,745	1,005	0.00	cJ47-114	l _N	21,414	222	0.00
	nM	14,043	751	0.00		nl	22,665	776	0.05
J47-114 _C	M _N	13,073	767	0.00	J47-114 _C	l _N	18,030	1,218	0.00
	nM	14,768	562	0.00		nl	18,985	420	0.00
NJ47-114	M1-25 _C	317,358	4,410	19.76	NJ47-114	J _C	80,475	4,808	0.19
	cM1-25	325,525	8,722	20.30		cJ	18,586	691	0.00
J47-114 _N	M1-25 _C	273,832	7,043	16.92	J47-114 _N	J _C	19,376	877	0.00
	cM1-25	267,785	13,573	18.01		cJ	16,051	1,144	0.00
cJ47-114	M1-25 _N	291,753	14,128	18.09	cJ47-114	J _N	15,770	699	0.00
	nM1-25	273,405	8,627	16.89		nJ	15,141	535	0.00
J47-114 _C	M1-25 _N	306,459	7,336	19.05	J47-114 _C	J _N	15,060	1,128	0.00
	nM1-25	291,715	5,997	18.09		nJ	16,024	1,276	0.00
NJ47-114	M60-84 _C	303,601	20,553	20.55	NJ47-114	K _C	101,494	3,641	3.67
	cM60-84	327,115	5,204	16.84		cK	20,158	881	0.00
J47-114 _N	M60-84 _C	295,661	12,051	19.99	J47-114 _N	K _C	19,932	661	0.00
	cM60-84	324,978	14,970	16.72		cK	16,395	702	0.00
cJ47-114	M60-84 _N	305,845	4,929	20.71	cJ47-114	K _N	12,912	694	0.00
	nM60-84	298,693	14,978	20.20		nK	15,697	686	0.00
J47-114 _C	M60-84 _N	289,178	29,570	19.53	J47-114 _C	K _N	15,915	483	0.00
	nM60-84	287,088	7,985	19.38		nK	16,293	441	0.00
GvpJ47-114 interactions with accessory Gvp					NJ47-114	L _C	320,314	10,263	9.68
NJ47-114	F _C	16,562	860	0.00		cL	24,013	565	0.00
	J47-114 _N	cF	14,278	2,020	0.00	L _C	46,604	2,951	0.55
F _C		13,824	609	0.00	cL	20,888	361	0.00	
cJ47-114	F _N	13,240	510	0.00	J47-114 _C	L _N	112,470	92,340	3.87
	nF	13,166	442	0.00		nL	24,841	411	0.06
J47-114 _C	F _N	16,247	520	0.00	L _N	20,225	481	0.00	
	nF	15,313	716	0.00	nL	20,794	674	0.00	
GvpJ1-56 interactions with accessory Gvp					NJ1-56	F _C ¹	81,078	24,236	0.15
NJ47-114	G _C	331,093	40,997	14.61		cF ¹	108,337	4,428	0.33
	J47-114 _N	cG	22,518	878	0.06	J1-56 _N	F _C ¹	66,713	21,996
G _C		21,539	3,383	0.08	cF ¹		76,244	8,684	0.02
cJ47-114	G _N	16,476	1,223	0.00	cJ1-56	F _N ¹	99,538	8,033	0.23
	nG	13,251	408	0.00		nF ¹	101,754	9,478	0.25
J47-114 _C	G _N	14,915	601	0.00	J1-56 _C	F _N ¹	108,050	6,702	0.33
	nG	11,579	538	0.00		nF ¹	95,692	16,835	0.19

continued

transformant		LAU/mm ²	σ LAU/mm ²	Relative fluorescence (rf)	transformant		LAU/mm ²	σ LAU/mm ²	Relative fluorescence (rf)
NJ1-56	G _C ¹	116,329	5,191	0.43	NJ1-56	J _C	21,523	352	0.17
	cG ¹	100,934	8,438	0.24		cJ	12,922	1,854	0.00
J1-56 _N	G _C ¹	95,945	2,425	0.18	J1-56 _N	J _C	21,410	1,938	0.17
	cG ¹	81,555	21,073	0.13		cJ	14,624	1,355	0.00
cJ1-56	G _N ¹	110,939	8,529	0.37	cJ1-56	J _N	18,658	542	0.02
	nG ¹	94,514	6,221	0.17		nJ	18,531	1,072	0.03
J1-56 _C	G _N ¹	101,179	3,363	0.25	J1-56 _C	J _N	16,474	629	0.00
	nG ¹	103,467	2,950	0.28		nJ	17,288	1,587	0.01
NJ1-56	H _C ¹	85,957	15,050	0.12	NJ1-56	K _C ¹	107,987	1,078	0.37
	cH ¹	73,801	4,650	0.00		cK ¹	104,292	3,765	0.32
J1-56 _N	H _C ¹	85,002	4,650	0.05	J1-56 _N	K _C ¹	94,576	12,779	0.20
	cH ¹	69,432	14,878	0.02		cK ¹	83,818	4,811	0.07
cJ1-56	H _N ¹	109,585	3,404	0.35	cJ1-56	K _N ¹	115,008	8,435	0.46
	nH ¹	103,801	6,612	0.28		nK ¹	64,917	2,803	0.00
J1-56 _C	H _N ¹	101,667	3,018	0.25	J1-56 _C	K _N ¹	105,270	11,477	0.34
	nH ¹	126,969	18,753	0.57		nK ¹	72,108	29,437	0.14
NJ1-56	l _C ¹	97,928	7,984	0.21	NJ1-56	L _C ¹	105,270	3,549	0.34
	c ¹	97,230	4,010	0.20		cL ¹	111,793	11,823	0.42
J1-56 _N	l _C ¹	79,291	28,865	0.17	J1-56 _N	L _C ¹	84,466	7,502	0.08
	c ¹	80,563	30,553	0.18		cL ¹	118,065	18,329	0.35
cJ1-56	l _N ¹	119,173	6,352	0.51	cJ1-56	L _N ¹	111,356	7,800	0.27
	n ¹	91,665	7,211	0.16		nL ¹	119,482	6,313	0.37
J1-56 _C	l _N ¹	114,719	5,457	0.46	J1-56 _C	L _N ¹	100,725	3,806	0.15
	n ¹	106,568	15,370	0.35		nL ¹	95,150	2,439	0.09

Supplementary Table S5. Split-GFP analyses investigating interactions of GvpF and GvpL fragments. The fluorescence was measured in LAU/mm² and the relative fluorescence calculated.

transformant		LAU/mm ²	σ (LAU/mm ²)	Relative fluorescence (rf)	transformant		LAU/mm ²	σ (LAU/mm ²)	Relative fluorescence (rf)
GvpL fragments					nL133-281	K _C	100,620	13,627	6.15
					cK		19,790	1,166	0.41
nL1-134	G _C	50,142	3,124	2.63	L133-281 _N	K _C	64,662	2,487	3.60
	cG	14,096	266	0.02		cK	19,408	1,092	0.38
L1-134 _N	G _C	16,343	1,129	0.18	cL133-281	K _N	10,856	2,638	0.00
	cG	15,053	1,148	0.09		nK	14,461	886	0.08
cL1-134	G _N	13,412	387	0.00	L133-281 _C	K _N	18,570	2,518	0.32
	nG	12,496	620	0.00		nK	29,739	571	1.11
L1-134 _C	G _N	12,312	317	0.00					
	nG	15,944	2,165	0.16	nL133-281	M _C	36,895	900	1.62
					cM		10,323	949	0.00
nL1-134	J _C	34,069	573	0.97	L133-281 _N	M _C	16,662	560	0.18
	cJ	16,779	1,566	0.03		cM	10,619	811	0.00
L1-134 _N	J _C	15,699	1,027	0.00	cL133-281	M _N	14,825	892	0.00
	cJ	15,669	653	0.00		nM	10,023	871	0.00
cL1-134	J _N	11,821	1,168	0.00	L133-281 _C	M _N	14,410	711	0.00
	nJ	15,406	2,638	0.14		nM	13,218	2,063	0.00
L1-134 _C	J _N	10,553	1,233	0.00					
	nJ	12,335	825	0.00					
GvpF fragments									
nL1-134	K _C	53,021	3,032	2.84	nF1-110	A _C	21,277	1,773	0.39
	cK	14,979	405	0.08		cA	17,132	1,592	0.12
L1-134 _N	K _C	15,034	392	0.09	F1-110 _N	A _C	20,238	1,677	0.32
	cK	13,236	397	0.00		cA	18,346	1,568	0.20
cL1-134	K _N	12,589	1,053	0.04	cF1-110	A _N	20,836	206	0.36
	nK	13,249	381	0.00		nA	20,129	912	0.32
L1-134 _C	K _N	14,506	648	0.05	F1-110 _C	A _N	18,209	1,457	0.19
	nK	13,432	1,679	0.05		nA	20,154	1,399	0.32
nL1-134	M _C	23,506	4,905	0.36	nF1-110	A1-22 _C	21,403	931	0.40
	cM	12,265	1,518	0.00		c A1-22	20,632	636	0.35
L1-134 _N	M _C	12,682	581	0.00	F1-110 _N	A1-22 _C	21,243	576	0.39
	cM	11,926	968	0.00		c A1-22	20,265	2,809	0.32
cL1-134	M _N	11,619	585	0.00	cF1-110	A1-22 _N	21,316	483	0.39
	nM	11,598	318	0.00		n A1-22	17,377	1,284	0.14
L1-134 _C	M _N	11,452	1,077	0.00	F1-110 _C	A1-22 _N	20,320	1,597	0.33
	nM	12,740	1,072	0.00		n A1-22	24,176	831	0.58
nL133-281	G _C	48,561	3,402	1.82	nF109-221	A _C	13,664	1,480	0.06
	cG	13,734	378	0.00		cA	10,712	575	0.00
L133-281 _N	G _C	27,798	1,177	0.56	F109-221 _N	A _C	12,824	704	0.01
	cG	14,342	380	0.00		cA	12,280	632	0.00
cL133-281	G _N	11,212	1,064	0.00	cF109-221	A _N	9,574	372	0.00
	nG	12,734	1,213	0.00		nA	10,993	449	0.00
L133-281 _C	G _N	14,209	325	0.00	F109-221 _C	A _N	14,080	3,218	0.13
	nG	16,994	2,105	0.04		nA	15,133	2,250	0.15
nL133-281	J _C	30,374	1,789	0.71	nF109-221	A1-22 _C	17,370	773	0.30
	cJ	13,146	735	0.00		cA1-22	9,356	726	0.00
L133-281 _N	J _C	20,040	1,263	0.13	F109-221 _N	A1-22 _C	12,889	244	0.00
	cJ	12,166	613	0.00		cA1-22	11,514	471	0.00
cL133-281	J _N	12,221	447	0.00	cF109-221	A1-22 _N	10,052	1,781	0.00
	nJ	12,786	603	0.00		nA1-22	14,719	413	0.10
L133-281 _C	J _N	14,866	612	0.00	F109-221 _C	A1-22 _N	11,203	910	0.00
	nJ	13,679	327	0.00		nA1-22	53,953	8,730	3.03