

Supplemental Figures and Table for:

SS Kharade and MJ McBride. "The *Flavobacterium johnsoniae* chitinase ChiA is required for chitin utilization and is secreted by the type IX secretion system"

1 MKHYRLLFLLLFPLLASAQPAGHKK**VVGYAQWSIYARDFNVPK**IDGSK
 51 **LTHLNYSFYGTTYDPAHPENTK**LK**CLDTYADFEHMEGGIPWDAPVKGNFY**
 101 **DLMKLQKQYPHLKILISVGGWTKGQDLSPIAASPVAR**AALAADMANFIVT
 151 YPFIDGFDIDWEYPLSGGTDGTEIVNGMPVPPQ**KYSPDDNKNLVLLLKAM**
 201 **RQAMPNKLVTIAAGNNVR**NVSKQYLGPNNAQYGMTEDISTYCDYITYFG
 251 YDFGGNWDY**KTCYNAPLYASGNPNPLYGATQSESLDELTNQYLVNIGFP**
 301 **ANKLIMGLPFYGGKFDNVAANSTNGLFVAAPRYIVPGCTNPQNPTGTWDG**
 351 **SGACEKSGSIEICDLVGNPVTNSHAYLDPNTMMVTPSAASAGWVRYFDNT**
 401 **TKVPYLYNSTLQKFISYEDKQSMDLKVQYIKSRNLAGGMIWELSQDTRGS**
 451 **IPNSLLNQVDTSPGVSVPPTVSI**SGSVKNGS**ALVTDVTVELRNASNAVIQ**
 501 **TVVSANGNFANNLTSGQNYSLTALK**ATYTFTPVTLVNVTVNQTAVVING
 551 TQPTYTVSGTVLDGSTPVSGVTVTAVSGSTTLTAVSNASGVYSIAGLTAG
 601 LNFTVTAAKS**GFSYAPASTVYNAIDSNK**TLNFTQGAPVVNYTVSGTVLNS
 651 TTPVSGVTVTASF TGGSYAAVTNASGTYSLSLPSGGNYTVTAALTGQFTT
 701 PASTVYSNLNANK**TLNFTQDVVSTSKISGTVKNGTNPVAGAK**VELVLPW
 751 TDNTHNWK**SVIATTTDAQGKYSFDNSVVDGYTQVLSLKLNSWQNGEVAYYP**
 801 **NNLANFAVPANPTVYFNFTSSTAK**SALAAAANLISGTVKNGTTPVAGAKV
 851 **EIVLPWTDNTHNWK**SVLATTDASGNYSFDNSV VAGYTQILSLKLNKWENG
 901 DVTYYPNNLANFAVPTTPTIYNFNQAVVATKPVVTITAPTASAIAINLG
 951 SAINFVASVGLSAVDATTISSVVFSLDGQSLSTANSSGTYTAAWTPAANQ
 1001 FLSLHTLTVTATASNGTTDSKTYSFTLTCSGANCPNALPVITWNSPSNTT
 1051 VYQNTFQVVPISVTAVSDGTVSGVTITINGGTFNMTAGTNNTYTYNFTP
 1101 SAYQDYPVVIKATDNKSGVTTLNNTIKIATVSTNRFIPLPSKIILGYAHS
 1151 WENAGAPFLYFSQMVGSKFNVDYSFVETVNRDGYTPILTTNDTRYLTNG
 1201 VFNKQLLKNDIKSLRDSGVPVIVSIGGQNGHVLDNVTQKNI FVNGLKAI
 1251 IDEYQFDGVDIDFEGGSMNMFNAGGLRDISYAGISAYPRLKKNVDAFKELK
 1301 AYYGPGFLLTAAPETQYVQGGYTTYTDTFGSFLPIIQNLRNELDLLAVQL
 1351 YNTGGENGLDGQYYGTAKKSNMVTAL TDMVIKGYNIAS TGMRFDGLPASK
 1401 VLIALPACPSAAGSGYLTPTEGINAMHYLRGTTFSGR TYTMQPGGPYPS
 1451 LRGLMTWSVNWDASSCGNSSELSKAYAA YFASQTAAKTLVLDLDISAKSNA
 1501 TIAYFKNNALSVTNENEDIAQVDVFNVLGQNLVSHRNVQNNKEVLLHNQS
 1551 FSSKQLFLVVVTDKAGNKKSFKVMNFLN

Figure S1. The approximately 92 kDa secreted form of ChiA corresponds to the amino-proximal portion of the full length ChiA. Cell-free spent medium from the *chiA* mutant CJ1808 complemented with pSSK05 which carries *chiA* was separated by SDS-PAGE and proteins were detected by silver staining. The approximately 92 kDa band was cut from the gel shown in Figure 5 and subjected to LC-MS/MS analysis. The primary amino acid sequence of ChiA is shown, with the regions detected by LC-MS/MS highlighted in red. 131 spectral matches were detected to ChiA. All of these fell between amino acids 27 and 864, which corresponds to the amino-proximal region of the protein after removal of the signal peptide.

1 MKHYRLLFLLLFPLLASAQPAGHKKVVGYYAQWSIYARDFNVPKIDGSK
 51 LTHLNYSFYGTTYDPAHPENTKCLKLDTYADFEHMEGGIPWDAPVKGNFY
 101 DLMKLLKQKYPHLKILISVGGWTKGQDLSPIAASPVARAALAADMANFIVT
 151 YPFIDGFDIDWEYPLSGGTDGTEIVNGMPVPPQKYSRDDNKNLVLLLKAM
 201 RQAMPNKLVTIAAGNNVRNVSKQYLGPNNRAQYGMTEDISTYCDYITYFG
 251 YDFGGNWDYKTCYNAPLYASGNPNDPYLGATQSESLDELTNQYLNVIIGFP
 301 ANKLIMGLPFYGGKFDNVAANSTNGLFVAAPRYIVPGCTNPQNPTGTWDG
 351 SGACEKSGSIEICDLVGNPVTNSHAYLDPNTMMVTPSAASAGWVRVYDNT
 401 TKVPYLYNSTLKKQFISYEDKQSMCLKVQYIKSRNLAGGMIWELSQDTRGS
 451 IPNSLLNQVDTSPGVSVPPTVSI S GSVKNGSALVTDVTVELRNASNAVIQ
 501 TVVSANGNFANLNTSGQNYSLTALKATYTFPTVTLVNVTVNQTAVVING
 551 TQPTYTVSGTVLDGSTPVSGVTVTAVSGSTTLTAVSNASGVYSIAGLTAG
 601 LNFTVTAAKSGFSYAPASTVYNAIDSNKTLNFTQGAPVVNYTVSGTVLNS
 651 TTPVSGVTVTASF TGGSYAAVTNASGTYSLSLPSGGNYTVTAAL TQTF
 701 PASTVYSNLNANKTLNFTQDVVSTSKISGTVKNGTNPVAGAKVELVLPW
 751 TDNTHNWKSVIATTTDAQKYSFDNSVVDGYTQVLSLKLNSWQNGEVAYYP
 801 NNLANFAV PANPTVYNFNTSSTAKSALAAAANLISGTVKNGTTPVAGAKV
 851 EIVLPWTDNTHNWKSVLATT DASGNYSFDNSV VAGYTIQLSLKLNWENG
 901 DVTYYPNNLANFAVPTTPTTIYFNFRQAVVATKPVVTITAPTASAIAINLG
 951 SAINFVASVGLSAVDATTISSVVFSLDGQSLSTANSSGTYTAAWTPAANQ
 1001 FLSHTLTVTATASNGTTDSKTYSFLLTCSGANCPNALPVITWNSPSNTT
 1051 VYQNTFQVVPISVTAVDS DGT VSGVTITINGGTFNMTAGTNNTYTYNFTP
 1101 SAYQDYPVVIKATDNKSGVTTLNNTIKIATVSTNRFIPLPSKIIILGYAHS
 1151 WENAGAPFLYFSQMVGSKFNVVDYSFVETVNRDGYTPIILTTNDTRYLTNG
 1201 VFNKQLLKNDIKSLRDSGVPVIVSIGGQNGHVLDNVTQKNIFVNLKAI
 1251 IDEYQFDGVDIDFEGGSMNFNAGGLRDISYAGISAYPRLKNVVDAFKELK
 1301 AYYGPGFLLTAAPETQYVQGGYTYTDTFGSFLPIIQNLRNELDLLAVQL
 1351 YNTGGENGLDGQYYGTAKKSNMVTAL TDMVIKGYNIAS TGMRF DGLPASK
 1401 VLIALPACDSAAGSGYLTPTTEGINAMHYLR TGTTFSGR TYTQMOPGPYPS
 1451 LRGLMTWSVNWDASSCGNSSELSKAYAAYFASQTAAKTLVLDDISAKSNA
 1501 TIAYFKNNALSVTNENEDIAQVDVFNVLGQNLVSHRNVQNNKEVLLHNQS
 1551 FSSKQLFLVVVTDKAGNKKSKFKVMNFLN

Figure S2. The approximately 65 kDa secreted form of ChiA appears to correspond to the carboxy-proximal portion of full length ChiA. Cell-free spent medium from the *chiA* mutant CJ1808 complemented with pSSK05 which carries *chiA* was separated by SDS-PAGE and proteins were detected by silver staining. The approximately 65 kDa band was cut from the gel shown in Figure 5 and subjected to LC-MS/MS analysis. The primary amino acid sequence of ChiA is shown, with the regions detected by LC-MS/MS highlighted. Regions in red correspond to sequences for which multiple (2 to 7) spectral matches were obtained, and regions in orange correspond to sequences for which single spectral matches were obtained, and which were thus apparently less abundant. In total, 79 spectral matches were detected to ChiA. 77 of these fell between amino acids 850 and 1487, which corresponds to the carboxy-proximal region of the protein immediately upstream of the C-terminal region involved in targeting to the type IX secretion system. The 2 spectral matches to sequences at positions 315 to 332 and 449 to 478 probably correspond to breakdown products of the 92 kDa amino-proximal portion of ChiA. No spectral matches were detected to the carboxy-terminal 91 amino acids suggesting that this region was removed by proteolysis during secretion.

SprB - - I T V R D K N G C T D T K D Y Y I E Y V - - - - D V C L D N Y F F T P N G D G V N D T W G P G C - - - T N - I Y N H L K F S I 6443
 Fjoh_1123 - - V Y L R D K E G C G Q D S K E V T - - - - V I D Y P K F F T P N N D G G Y N D F W H I K N - - - T S - K F P N S K I S I 1045
 Fjoh_1645 - - V V V E D N S D Y E N I D G D K P T V L D L N G C K I K V F L N A F F T P N N G D Q K N E R F Y I Q G - - - L E - C Y P P E N T V T I 2421
 Fjoh_1720 - - A Y V V K E K N G C G G I G L D F V - - - - V L V F P A F F T P N N D S Y N D L W E Y T G - - - M E - N Y P Q A Q V E T I 673
 Fjoh_1985 - - V V F T N K E G C Q A E D S V T I T V I P L E - - K D E T K Y G F F S P N G D G I N D S F W E I D K - - - I T - D Y P P E N E V L I 2000
 Fjoh_2273 - - - - A L T D C D N V P N D F F - - - - - I P D G F F S P N G D G I N D S F W E I K D - - - I E F L Y P P N Y T L E I 811
 Fjoh_3478 - - - P L D V D A A N N S A S A S V E P I - - - - C L T V Y E F F T P N N D G A N D L F R Y I R G C - - - I E - S Y P P N N E L K V 3678
 Fjoh_3952 - - I K V E D K S D A A N N E N G D A P T E I D V N G C K I K I F N A I S L N G D N M N E R F Y I R G - - - I E - C Y P P D N T V Q I 3125
 Fjoh_3971 - - - Q I D T N S S N N Q D S A A V S P N - - - - C L K I Y N E F F S P N D D G Q N D T F Y I D C - - - I T - Q Y P P D N Q L E I 3548
 Fjoh_4538 I L L T M E V N D D C K V L A C E - - - - N I L V H N A F F S P N G D G K N D V F L I D G I G D L T - C Y P P E N T V E I 2518
 Fjoh_4750 I K Q T Y Y P F G C Q Y S Y S A T T K V E K - - G Y S L I M P N A F F T P N N D G Y N D T F A P - - - V F L G L S D I T L D V 1406
 Fjoh_4934 - - A I A K D K N G C G L S N S F L L Y - - - - V L D Y P R F F T P N G D G Y N D L W V I E D - - - S N - V L P N Y T I H I 722
 ChiA - - - A S S C G N S S E L S K A Y A A Y F A S Q T A A K T L V L D D I S A K S N A T I A Y F K N N A L S V T N E N E D I A Q V D V 1524

SprB F D R Y Y G K V I A K - Y T Y G Q K - - - W D G R Y - - - - - N G E E L P S G D Y W Y V L K L N D E N D G - - - - R E F - V G 6491
 Fjoh_1123 F D R Y Y G K L I K E L F A N D H G - - - W D G F Y - - - - - N G S Q M P A D D Y W F K A N F N E N - - - - I N F - S G 1091
 Fjoh_1645 Y N R W Y G V L I F D V D H Y N N V D R V F K G Y S F G R T T M K Q S E G L P A V G T Y F Y I L K Y K D S D S N - - - - P H E T - S G 2481
 Fjoh_1720 F D R Y Y G K L I A Q L N A S K M S - - - W D G T F - - - - - E K T P M P A S D Y W Y A L K T I D D S - - - - - K P I L R G 720
 Fjoh_1985 Y S R W G D L V Y Q T K G Y D N S T N V F S G I A N K S R N L - G A S Q L P E G T Y F F E I R V N Q P H H F - - - - - K K L - K G 2058
 Fjoh_2273 F N R Y Y G N G M Y K G D K N K P A - - - W D G M N Y E K S G I - A G V A P N G V Y F Y V L H F N K D N K P - - - - - P K - Q G 865
 Fjoh_3478 F N R Y Y G A L V Y S K Q H Y E N D - - - W D G T A N V S G V V N R G D M L P T G T Y F Y V I T I G D G T V K - - - - - K G 3731
 Fjoh_3952 F N R W Y G V L V F E R D H Y N N N D I V F K G F S E G R T V V K E S N G L P E G T Y Y Y I V R Y K D N N S N - - - - P K Q E - A G 3185
 Fjoh_3971 E N R W G N L V Y Y K K G Y D N T - - - W D G K A D G - - - - S A K T L P E G T Y F Y V L D L - - G N G S - - - - - A K K - S G 3597
 Fjoh_4538 Y N R W Y G I L V F E T H N N N T T N A F G D T S R G R T T I R Q S E G L P T G T Y F Y I V T Y K S V D G N N V I Q N N K K - E G 2582
 Fjoh_4750 F D T W G G V V I Y T E K G T N I R G - - - W N G K V - - - - - K D I D A E N G N Y Y Y K I I L K T F Y N H - - - - - T I V E K G 1457
 Fjoh_4934 F D R Y Y G K F L K E M N Q N S P G - - - W N G L F - - - - - N G Q Q L P S D D Y W F T L T F A D G - - - - - R N V - K G 768
 ChiA F N V L G Q N L V S H R N V Q N N - - - - K E V L L - - - - - H N Q S F S S K Q L F L V V V T D K A G N K - - - - - K S F - - K 1572

SprB H F T L Y R 6497
 Fjoh_1123 H F S L K R 1097
 Fjoh_1645 Y L Y I N K 2487
 Fjoh_1720 H F S L K R 726
 Fjoh_1985 Y L V L K R 2064
 Fjoh_2273 R L Y L N R 871
 Fjoh_3478 W L S I M R 3737
 Fjoh_3952 Y L Y L I K 3191
 Fjoh_3971 W L Y L K - 3602
 Fjoh_4538 Y L Y L S K 2588
 Fjoh_4750 A F T L I K 1463
 Fjoh_4934 H F S L K R 774
 ChiA V M N F L N 1578

Figure S3. Alignment of the C-terminal domain (CTD) of ChiA with the CTDs of *F. johnsoniae* TIGR04131 family members using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. TIGR04131 is described as ‘gliding motility-associated C-terminal domain’ on the TIGRFAM website (<http://www.jcvi.org/cgi-bin/tigrfams/HmmReportPage.cgi?acc=TIGR04131>). TIGR04131 family members were identified by searching the *F. johnsoniae* genome using the IMG v 4.0 Function Profile Tool. As shown, the ChiA CTD has little if any similarity to the CTDs of TIGR04131 family members.

Alignment of the CTD of ChiA with the CTDs of *F. johnsoniae* TIGR04183 family members

Fjoh_0074	V	K	A	Y	F	N	P	T	-	S	D	V	I	N	F	T	V	K	T	-	-	N	E	S	-	K	N	L	K	L	R	-	-	L	Y	D	L	-	N	G	R	A	L	G	N	P	I	D	I	Q	S	S	E	E	V	N	T	T	V	M	S	L	-	-	-	1106
Fjoh_0547	L	S	L	Y	F	N	P	V	V	N	G	K	V	Y	I	S	S	K	N	-	-	-	D	L	E	K	E	I	I	-	-	V	F	D	I	-	L	G	K	K	V	L	Q	A	-	H	L	T	T	K	E	-	-	-	L	N	V	-	-	-	86					
Fjoh_0549	I	T	V	I	P	V	T	F	V	N	P	F	-	A	K	T	V	Q	S	N	V	N	V	-	E	D	S	I	Q	P	Y	S	I	N	-	-	V	Y	N	F	-	E	G	Q	K	V	L	T	K	-	E	V	K	S	I	E	E	N	K	L	S	L	D	526		
Fjoh_0707	V	Y	V	Y	F	N	P	V	-	R	P	T	Y	S	G	T	V	K	V	-	-	A	G	L	I	D	K	A	N	I	K	-	-	I	T	D	J	-	E	G	N	L	V	Y	E	T	-	T	S	D	G	G	T	I	E	W	D	T	T	A	F	-	-	-	729	
Fjoh_0798	F	V	L	Y	F	N	P	N	-	K	G	S	F	T	V	Q	F	K	S	-	-	-	E	S	T	S	V	K	V	F	-	-	V	N	D	I	-	L	G	K	T	I	Y	A	K	-	T	F	E	T	D	G	D	-	-	-	F	N	Q	N	I	-	-	-	858	
Fjoh_0808	G	L	L	I	S	-	-	V	K	D	K	T	I	K	V	T	S	A	K	-	-	-	E	N	I	K	E	V	N	-	-	I	F	D	I	-	T	G	K	L	I	Y	N	K	-	-	K	K	V	G	N	-	-	T	E	L	S	I	-	-	-	1407				
Fjoh_0848	F	Y	V	F	F	N	P	A	-	Q	T	T	I	T	V	E	N	L	N	-	-	-	S	K	N	F	D	F	E	-	-	F	F	N	F	-	E	S	K	S	V	L	K	G	-	-	-	K	T	S	D	-	-	G	T	I	N	I	-	-	-	443				
Fjoh_0886	F	A	L	Y	F	N	P	V	-	E	S	E	L	N	V	T	V	S	E	-	-	-	E	N	A	Y	S	Y	K	-	-	I	I	N	A	-	L	G	Q	Q	L	G	S	G	-	-	Q	V	S	G	A	-	-	-	I	D	V	-	-	-	898					
Fjoh_1022	V	T	L	Y	F	N	P	S	-	P	D	R	I	T	N	V	A	P	Q	-	-	-	-	Q	S	T	I	S	-	-	V	I	S	P	-	S	G	S	V	I	Y	Q	K	-	-	K	T	S	E	N	-	-	T	E	I	N	L	-	-	-	438					
Fjoh_1188	V	V	I	H	F	N	P	T	-	K	G	E	L	N	I	K	N	V	N	-	-	-	-	L	E	K	A	N	-	-	V	Y	N	V	-	L	G	Q	L	V	K	S	F	T	L	N	S	N	T	D	-	-	N	T	I	N	L	-	-	-	1363					
Fjoh_1189	V	V	I	H	F	N	P	T	-	K	G	E	L	N	I	K	N	V	N	-	-	-	-	L	E	K	A	N	-	-	V	Y	N	V	-	L	G	Q	L	V	K	S	F	T	L	N	S	N	T	D	-	-	N	T	I	N	L	-	-	-	1653					
Fjoh_1208	I	S	I	Y	F	N	P	S	V	N	N	E	F	N	V	L	P	E	-	-	L	E	S	G	D	M	A	S	I	S	-	-	V	S	D	J	-	N	G	R	T	V	L	T	E	-	-	R	L	S	S	S	-	-	G	K	I	D	H	-	-	-	1023			
Fjoh_1231	P	F	L	Y	F	N	P	V	-	S	G	T	L	Y	L	S	D	Q	N	-	-	-	Q	K	V	E	K	V	Q	-	-	I	Y	N	V	-	L	G	V	L	V	K	T	S	-	-	-	Q	K	G	N	-	-	E	S	I	D	L	-	-	-	893				
Fjoh_1269	F	R	Y	Y	F	N	P	V	-	H	V	L	N	I	S	N	A	S	-	-	-	N	I	D	E	V	E	-	-	V	I	S	V	-	S	G	K	S	V	I	L	V	K	-	Q	I	N	N	T	H	-	-	S	E	I	D	L	-	-	-	848					
Fjoh_1408	V	N	L	Y	F	N	P	V	-	S	N	H	F	T	L	S	T	A	V	-	-	-	-	S	K	V	Q	-	-	I	Y	S	V	-	S	G	Q	F	V	K	S	F	-	-	A	S	N	G	N	-	-	V	D	F	Q	F	-	-	-	928						
Fjoh_1905	M	A	V	Y	I	D	E	V	-	S	D	H	L	K	I	E	T	N	H	-	-	-	E	G	T	A	D	V	E	-	-	I	F	N	J	-	N	G	Q	S	V	L	K	R	N	V	N	F	V	K	G	N	-	-	L	S	E	I	E	V	-	-	-	669		
Fjoh_2150	L	A	V	Y	F	N	P	V	-	K	N	T	L	N	L	S	Y	Q	D	-	-	-	K	I	D	E	V	I	K	-	-	I	F	N	V	-	L	G	Q	E	I	L	N	K	-	-	N	I	S	A	S	-	-	D	T	V	D	M	-	-	-	330				
Fjoh_2389	F	L	V	Y	F	N	P	T	-	K	S	N	I	S	F	L	F	D	N	-	-	-	E	T	A	S	V	S	-	-	I	Y	S	L	-	L	G	Q	K	L	I	E	K	-	-	Q	I	T	N	Q	N	-	-	P	V	L	S	V	-	-	-	507				
Fjoh_2456	F	K	V	W	F	V	P	T	-	N	G	N	E	S	V	L	D	N	-	-	-	E	I	E	K	A	D	L	K	-	-	I	Y	D	V	-	L	G	K	E	V	Q	K	R	N	I	N	G	K	T	T	E	-	-	-	N	J	H	L	-	-	-	897			
Fjoh_2666	I	E	A	I	F	N	P	A	-	V	T	Y	T	N	V	I	I	G	Y	-	-	-	D	F	T	E	G	T	A	S	-	-	V	I	D	I	-	L	G	R	I	L	Q	Q	F	-	-	S	I	N	S	R	T	-	-	V	P	V	D	L	-	-	-	531		
Fjoh_3203	V	S	I	Y	F	N	P	S	V	N	N	E	F	N	I	A	L	P	E	-	-	L	S	P	D	D	T	A	I	T	-	-	V	T	D	I	-	N	G	R	K	V	L	V	K	-	-	K	L	N	S	N	S	-	-	A	K	I	N	H	-	-	-	956		
Fjoh_3246	F	F	I	S	P	V	P	N	-	D	G	N	F	T	L	H	L	N	G	-	-	-	D	E	G	T	F	D	L	V	-	-	I	F	D	A	-	N	G	K	A	V	Y	K	Q	P	K	L	E	I	N	S	N	-	-	F	S	K	E	I	-	-	-	2732		
Fjoh_3296	F	F	I	A	Q	D	N	Y	-	N	Q	L	L	K	A	S	N	P	D	-	-	-	T	R	N	F	K	S	F	S	-	-	L	Y	D	J	-	S	G	K	K	V	L	F	K	N	N	L	G	T	F	E	Q	N	-	-	Y	S	F	S	T	-	-	-	568	
Fjoh_3324	I	I	V	Y	F	N	P	S	-	K	G	L	F	H	L	S	K	E	L	-	-	-	-	-	E	W	T	-	-	V	F	S	V	-	S	G	S	K	I	K	E	G	-	-	-	R	G	N	-	-	E	I	S	T	-	-	-	948								
Fjoh_3421	M	T	A	Y	F	N	P	A	-	I	D	E	L	S	L	V	N	D	-	-	-	D	I	L	D	D	L	S	Y	G	-	-	V	F	D	I	-	N	G	K	T	V	S	Q	N	-	-	L	K	V	T	T	S	E	-	-	T	R	V	S	M	-	-	-	136	
Fjoh_3731	I	E	A	I	F	N	P	A	-	V	T	Y	T	N	V	I	I	G	Y	-	-	-	D	F	T	E	G	T	A	S	-	-	V	I	D	I	-	L	G	R	I	L	Q	Q	F	-	-	S	I	N	S	R	T	-	-	V	P	V	D	L	-	-	-	525		
Fjoh_3777	Y	K	I	Y	F	N	P	S	-	S	N	I	I	N	I	N	L	A	D	E	N	Y	R	P	V	S	S	S	L	I	R	A	E	L	Y	N	J	-	S	G	D	L	K	S	A	V	-	-	T	I	K	N	H	T	-	-	A	Q	L	D	V	-	-	-	1137	
Fjoh_3855	C	Y	L	K	Q	N	P	V	-	Q	D	N	L	V	L	E	I	A	E	-	-	E	Y	K	N	E	E	T	L	K	-	-	I	Y	N	T	-	S	G	V	L	L	K	E	S	-	-	-	S	Y	R	P	-	-	E	G	L	-	-	-	258					
Fjoh_4051	A	K	L	Y	F	N	P	I	Q	T	G	K	A	I	T	V	E	A	D	F	P	Q	E	E	L	N	N	M	Q	I	S	-	-	L	Y	S	V	-	S	G	Q	L	I	K	T	V	-	Q	S	S	A	L	-	-	T	E	I	Q	L	P	-	-	-	2236		
Fjoh_4174	L	N	I	Y	F	N	P	V	-	S	D	V	L	S	F	T	D	V	-	-	-	-	T	G	G	K	I	N	-	-	I	I	D	S	-	Q	A	V	L	G	S	Q	-	-	-	N	A	A	E	-	-	N	S	L	N	V	-	-	-	931						
Fjoh_4175	L	T	V	Y	F	N	P	S	-	E	D	T	L	F	F	S	A	E	V	-	-	-	S	G	A	N	V	N	-	-	I	I	N	S	-	E	G	A	T	V	S	T	-	-	Q	K	A	N	D	N	-	-	S	I	N	V	-	-	-	515						
Fjoh_4176	L	N	V	Y	F	N	P	V	-	E	N	T	L	F	T	T	D	L	-	-	-	-	S	G	G	D	V	K	-	-	I	V	N	A	Q	S	-	N	G	T	V	L	S	K	-	-	-	K	N	G	N	-	-	-	S	I	D	V	-	-	-	858				
Fjoh_4177	F	A	V	Y	F	N	P	A	-	G	N	Y	I	Q	V	S	L	P	E	-	-	-	N	L	N	K	I	I	T	-	-	I	Y	D	N	-	S	T	L	M	L	Q	N	K	P	E	A	N	A	S	E	-	-	S	V	I	D	L	-	-	-	1306				
Fjoh_4242	F	F	I	H	F	T	L	I	-	G	K	N	E	E	L	F	I	E	A	-	-	-	P	K	E	Q	N	A	V	F	Y	-	-	L	Y	T	J	-	S	G	Q	N	T	I	T	S	P	L	I	S	L	T	N	S	-	-	I	T	L	N	T	-	-	-	879	
Fjoh_4436	I	V	V	Y	F	N	P	T	-	S	G	L	F	S	I	Q	I	K	R	-	-	-	P	K	S	A	K	A	T	V	C	-	-	I	Y	N	L	-	N	G	R	V	L	Q	K	R	N	I	I	F	S	E	E	R	-	-	Q	S	F	E	F	-	-	-	1144	
Fjoh_4721	F	S	I	Y	F	N	P	S	-	N	G	H	F	T	I	Q	L	K	D	-	-	-	S	N	E	T	S	N	I	E	-	-	I	I	S	I	-	L	G	Q	R	V	F	S	Q	-	-	K	N	S	L	N	-	-	-	S	S	I	N	V	-					

Fjoh_0074	R	-	-	N	L	N	A	G	L	Y	I	Y	T	L	S	E	N	N	K	V	V	-	-	Y	K	N	K	I	L	K	N	-	-	-	-	1133	
Fjoh_0547	S	-	-	D	L	V	P	G	V	Y	I	I	R	L	S	E	Q	N	A	T	-	-	-	A	T	R	K	L	T	I	R	-	-	-	-	112	
Fjoh_0549	-	-	-	S	L	K	S	G	I	Y	I	I	K	S	K	N	-	-	-	-	-	-	-	-	E	T	R	K	V	L	K	-	-	-	-	546	
Fjoh_0707	G	R	Y	K	V	S	S	G	V	Y	M	I	F	I	S	A	Q	D	G	S	E	T	K	V	K	V	M	I	I	R	-	-	-	-	760		
Fjoh_0798	F	L	P	N	A	A	S	G	L	Y	L	V	T	V	I	D	G	D	K	R	-	-	-	T	V	R	K	I	I	N	-	-	-	-	886		
Fjoh_0808	S	N	L	Q	S	A	D	Q	V	L	L	V	K	V	N	L	E	N	N	A	Q	-	-	-	I	T	R	K	V	I	F	K	-	-	-	1436	
Fjoh_0848	L	-	-	G	L	D	S	G	F	Y	I	L	K	T	T	I	G	E	T	V	-	-	-	E	T	F	K	V	I	K	E	-	-	-	-	469	
Fjoh_0886	S	-	-	R	L	S	T	G	I	Y	L	I	E	L	N	N	G	K	E	K	-	-	-	I	V	K	K	F	A	K	K	-	-	-	-	924	
Fjoh_1022	G	-	-	S	Q	S	S	G	I	Y	F	V	V	K	I	S	N	D	D	F	K	S	-	-	I	T	K	K	V	T	L	K	-	-	-	465	
Fjoh_1188	S	-	-	G	L	P	K	G	V	Y	Y	V	Y	L	I	N	Q	D	T	A	-	-	-	S	A	K	K	V	I	V	E	-	-	-	-	1389	
Fjoh_1189	S	-	-	G	L	P	K	G	V	Y	Y	V	Y	L	I	N	Q	D	A	A	-	-	-	S	A	K	K	V	I	V	E	-	-	-	-	1679	
Fjoh_1208	-	-	-	R	L	A	S	G	I	Y	I	V	N	I	V	S	K	E	Y	K	-	-	-	T	T	K	K	L	I	V	K	-	-	-	-	1048	
Fjoh_1231	G	-	-	S	L	A	S	G	T	Y	L	A	K	I	F	T	T	D	G	S	-	-	-	I	S	Q	T	I	V	K	K	-	-	-	-	919	
Fjoh_1269	S	-	-	S	V	S	S	G	L	Y	F	L	K	V	K	S	E	G	Q	S	-	-	-	K	T	I	K	I	V	K	K	-	-	-	-	874	
Fjoh_1408	G	V	S	E	L	Q	T	G	L	Y	I	V	K	A	S	D	E	N	G	K	I	-	-	-	Q	V	M	K	F	I	K	K	-	-	-	957	
Fjoh_1905	S	-	-	R	L	P	K	G	V	Y	I	V	R	V	N	D	G	A	G	S	-	-	-	Y	S	K	K	V	L	K	Q	-	-	-	-	695	
Fjoh_2150	S	-	-	Q	M	L	P	G	T	Y	I	A	K	I	S	A	N	N	I	V	-	-	-	Q	T	F	K	I	V	K	I	-	-	-	-	356	
Fjoh_2389	E	-	-	G	L	T	N	G	L	Y	F	Y	T	F	D	A	G	S	L	H	-	-	-	K	T	G	K	I	I	K	Q	-	-	-	-	533	
Fjoh_2456	-	-	-	R	E	K	G	V	Y	I	L	K	V	S	N	P	N	N	K	K	V	L	H	V	K	K	I	I	V	K	-	-	-	-	-	924	
Fjoh_2666	S	-	-	H	Y	A	E	G	I	Y	I	I	K	I	K	T	D	V	K	T	-	-	-	E	S	V	K	V	I	K	T	V	R	-	-	559	
Fjoh_3203	-	-	-	D	L	A	S	G	I	Y	V	V	T	I	H	S	N	A	L	N	-	-	-	I	S	K	K	L	I	V	K	-	-	-	-	981	
Fjoh_3246	K	T	H	L	R	A	S	G	V	Y	F	L	I	L	Q	N	A	D	K	S	-	-	-	Y	K	A	F	L	I	K	-	-	-	-	2760		
Fjoh_3296	S	-	-	G	L	S	H	G	V	Y	I	A	V	E	L	T	D	D	N	E	K	-	-	-	I	S	Q	K	V	I	I	S	N	S	R	N	599
Fjoh_3324	S	-	-	E	Q	A	S	G	I	Y	F	L	K	T	N	A	S	A	-	-	-	-	-	-	K	A	I	I	I	S	K	Q	-	-	-	-	972
Fjoh_3421	Q	-	-	G	L	N	Q	G	V	Y	F	L	V	T	N	K	N	S	K	N	I	-	-	-	K	T	F	K	I	I	K	K	-	-	-	-	163
Fjoh_3731	S	-	-	H	Y	A	E	G	I	Y	I	I	K	I	K	T	D	V	K	T	-	-	-	E	S	V	K	V	I	K	T	V	R	-	-	553	
Fjoh_3777	S	-	-	A	P	L	P	G	V	Y	F	L	R	I	N	V	D	G	K	T	-	-	-	E	S	H	Q	V	L	V	K	-	-	-	-	1163	
Fjoh_3855	S	-	-	D	L	S	Q	G	I	Y	F	V	L	S	V	N	N	N	G	A	S	-	-	-	K	K	I	K	F	T	K	K	-	-	-	-	284
Fjoh_4051	Q	-	-	T	I	E	S	N	I	L	M	V	V	L	E	T	P	N	V	K	-	-	-	K	S	F	K	V	I	V	K	-	-	-	-	2262	
Fjoh_4174	S	-	-	N	L	K	Q	G	I	Y	F	I	V	L	E	K	D	G	Q	K	-	-	-	T	I	K	R	F	I	K	K	-	-	-	-	957	
Fjoh_4175	S	-	-	G	L	K	S	G	I	Y	L	I	L	V	E	K	D	G	I	K	-	-	-	T	V	R	R	F	I	K	K	-	-	-	-	541	
Fjoh_4176	S	-	-	H	L	A	K	G	I	Y	L	I	V	F	E	K	D	G	K	Q	-	-	-	T	I	K	R	F	I	K	K	-	-	-	-	884	
Fjoh_4177	S	-	-	R	L	T	K	G	I	Y	I	L	N	F	K	S	D	Q	K	S	-	-	-	W	T	K	K	L	I	K	Q	-	-	-	-	1332	
Fjoh_4242	A	-	-	-	-	-	S	G	I	Y	I	Y	K	I	I	T	G	S	G	K	V	-	-	-	Q	T	G	K	I	A	I	F	-	-	-	-	903
Fjoh_4436	N	I	T	G	A	T	F	G	I	Y	L	I	R	V	D	C	L	E	G	M	-	-	-	T	Q	N	L	I	L	K	N	-	-	-	-	1172	
Fjoh_4721	N	-	-	N	I	Q	K	G	I	Y	I	V	R	I	T	Q	G	S	K	T	-	-	-	S	S	K	K	I	I	I	N	-	-	-	-	617	
Fjoh_4723	S	-	-	P	L	P	S	G	I	Y	I	V	K	I	E	K	D	S	K	T	-	-	-	T	I	K	K	I	I	I	N	-	-	-	-	614	
Fjoh_4948	-	I	P	S	L	T	T	G	V	Y	I	F	Q	I	T	Y	A	N	G	T	V	-	-	-	K	T	K	N	L	A	V	N	-	-	-	-	320
ChiA	S	-	-	-	S	K	Q	L	F	L	V	V	V	T	D	K	A	G	N	K	-	-	-	K	S	F	K	V	M	N	F	L	N	-	-	-	1578

Figure S4. Alignment of the C-terminal domain (CTD) of ChiA with the CTDs of *F. johnsoniae* TIGR04183 family members using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. TIGR04183 is described as ‘Por secretion system C-terminal sorting domain’ on the TIGRFAM website (<http://www.jcvi.org/cgi-bin/tigrfams/HmmReportPage.cgi?acc=TIGR04183>). TIGR04183 family members were identified by searching the *F. johnsoniae* genome using the IMG v 4.0 Function Profile Tool. Fjoh_2336, Fjoh_2338, Fjoh_2339, and Fjoh_3296 were eliminated because of poor matches to the consensus. As shown, the ChiA CTD has limited similarity to the CTDs of TIGR04183 family members.

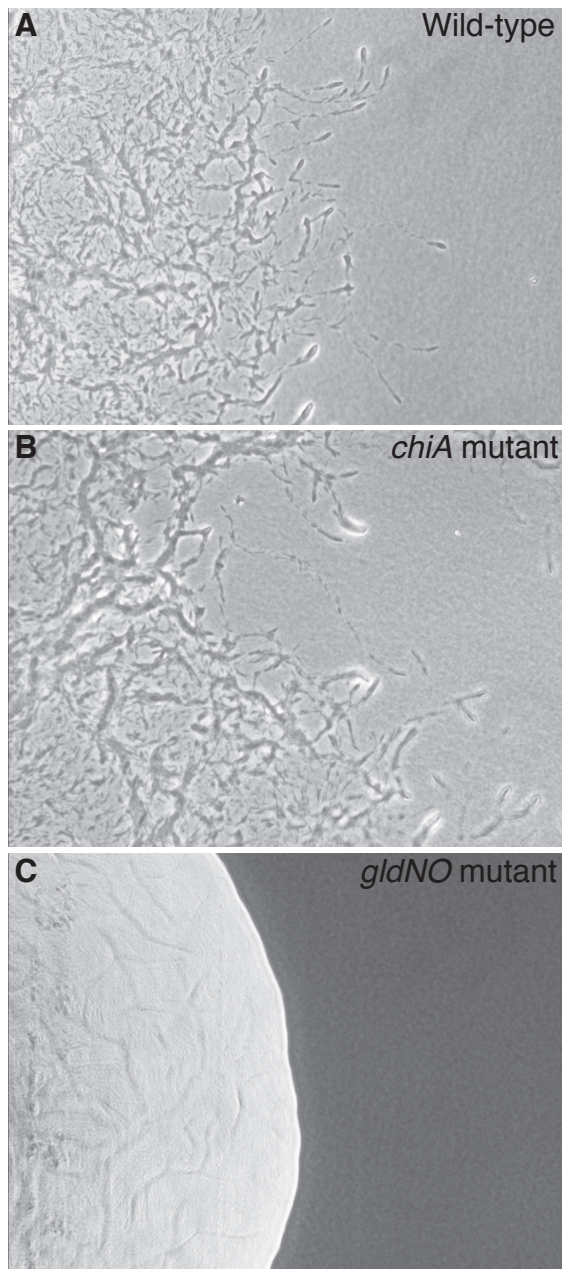


Figure S5. Disruption of *chiA* does not affect gliding motility. Colonies were grown for 42 h at 25°C on PY2 agar medium. Photomicrographs were taken with a Photometrics CoolSNAP_{cf}² camera mounted on an Olympus IMT-2 phase-contrast microscope. (A) Wild-type *F. johnsoniae* UW101. (B) *chiA* mutant CJ1808. (C) *gldNO* deletion mutant CJ1631A. Bar indicates 0.5 mm and applies to all panels.

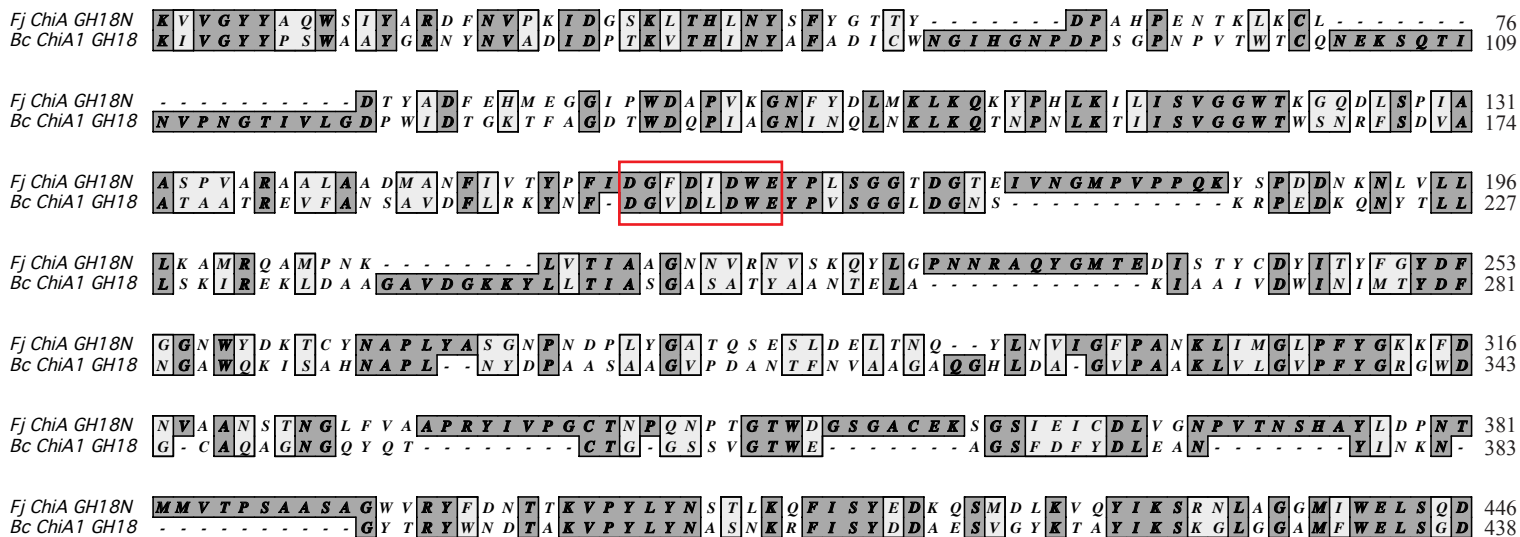


Figure S6. Alignment of *F. johnsoniae* ChiA_{GH18N} with the GH18 domain of *Bacillus circulans* ChiA1 using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. The conserved ‘DXXDXDXE’ GH18 catalytic site is boxed in red.

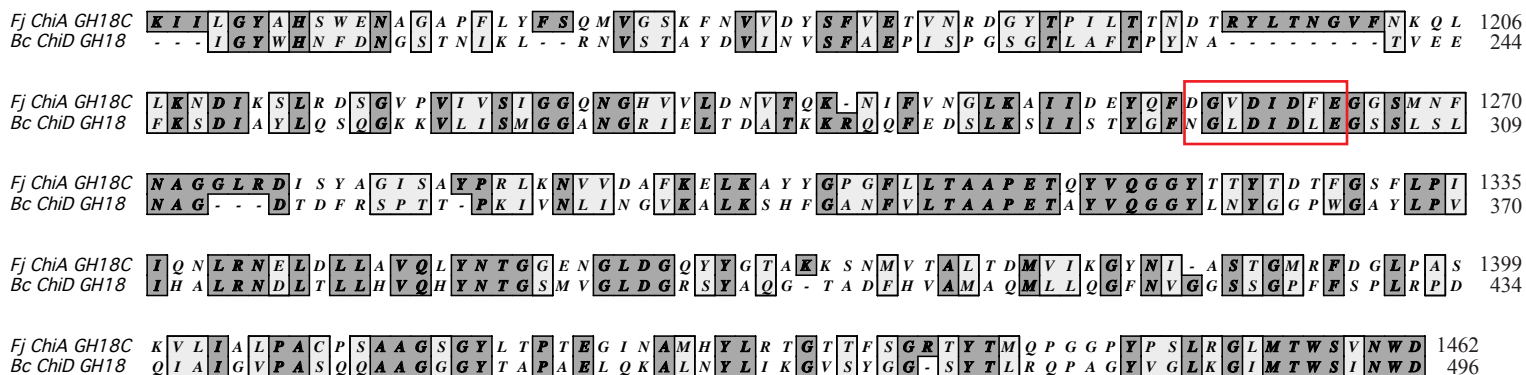


Figure S7. Alignment of *F. johnsoniae* ChiA_{GH18C} with the GH18 domain of *Bacillus circulans* ChiD using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. The conserved ‘DXXDXDXE’ GH18 catalytic site is boxed in red.

Table S1. Primers used in this study.

Primers	Sequence and Description
737	5'-AGGCACCCCAGGCTTTACT-3'; Reverse primer binding downstream of multiple cloning site of pLYL03.
862	5'-GCTAGGGATCCATGGTGAGCAAGGGCGAGG-3'; mCherry forward primer used in construction of pSSK30 and pSSK45; BamHI site underlined.
937	5'-GCTAGGGATCCTGATCCGTCAAGAACTGTTCCGC-3'; Reverse primer used in construction of pSAM1; BamHI site underlined.
938	5'-GCTAGGTCGACAGTCCGGTAGCAAGAGCTGCATTA-3'; Forward primer used in construction of pSAM1; Sall site underlined.
941	5'-TTGCACCTGCAACCGGATTTGTTC-3'; Reverse primer used for confirming and sequencing <i>chiA</i> disruption mutant CJ1808; Binds 532 bp upstream of primer 937.
974	5'-GCTAGTCTAGAGGTTTCATAATGCGCATCCTTAGGCA-3'; Reverse primer used to amplify <i>chiA</i> for construction of complementation plasmid pSSK05; XbaI site underlined.
975	5'-GCTAGGGATCCCTTCCAACCTGCAGTTGAGCGAAA-3'; Forward primer used to amplify <i>chiA</i> for construction of complementation plasmid pSSK05; BamHI site underlined.
1066	5'-GCTAGGGATCCAGTCCGGTAGCAAGAGCTGCATTA-3'; Forward primer used to amplify <i>chiA</i> for constructing pSSK07; BamHI site underlined.
1067	5'-GCTAGGTCGACTTTTGCACCTGCAACCGGATTTGTTC-3'; Reverse primer used to amplify <i>chiA</i> for constructing pSSK07; Sall site underlined.
1227	5'-GCTAGTCTAGATGCAGATCAGTCACCATCGCTTCA-3'; Forward primer used to amplify upstream region of Fjoh_4175 for constructing pSSK34; XbaI site underlined.
1228	5'-GCTAGGTCGACAGAAACAGAACCTCCTCCAAGCGA - 3'; Reverse primer used to amplify upstream region of Fjoh_4175 for constructing pSSK34; Sall site underlined.
1229	5'-GCTAGGTCGACTTCTTCTCGGCAGAAGTTTCGGGA- 3'; Forward primer used to amplify downstream region of Fjoh_4175 for constructing pSSK32; Sall site underlined.
1230	5'-GCTAGGCATGCTCCTAAAGTTGTTGTTCCGTTTGC-3; Reverse primer used to amplify downstream region of Fjoh_4175 for constructing pSSK32; SphI site underlined.
1266	5'-GCTAGTCTAGACTTGTACAGCTCGTCCATGCCG- 3'; Reverse primer to amplify mCherry for constructing pSSK30; XbaI site underlined.
1378	5'-GCTAGGGATCCGCAGTTCCTGCAAATCCAACAGTT- 3'; Forward primer to amplify the upstream region of <i>chiA</i> CTD for constructing pSSK26; BamHI site underlined.
1379	5'-GCTAGGTCGACAGATAATTCAGATGAATTACCGCAAGA-3'; Reverse primer to amplify the upstream region of <i>chiA</i> CTD for constructing pSSK26; Sall site underlined.
1380	5'-GCTAGGTCGACAATAATAAATGATTGAAAATTTAGAA -3'; Forward primer to amplify the region downstream of <i>chiA</i> for constructing pSSK27; Sall site underlined.
1381	5'-GCTAGGCATGCTGAAAATTTCCATTAGCCAGC-3'; Reverse primer to amplify the region downstream of <i>chiA</i> for constructing pSSK27; SphI site underlined.
1391	5'-TCTGGAAGAACATATACTATGCAGCCA- 3'; Forward primer used to confirm and sequence <i>chiA</i> CTD deletion.
1392	5'-TCACCTAATAACAATAACTAACCTC-3'; Reverse primer used to confirm and sequence <i>chiA</i> CTD deletion.
1404	5'-GCTAGGCATGCTCACCTAATAACAATAACTAACCTC-3'; Reverse primer to amplify <i>chiA</i> CTD for making construct pSSK52; SphI site underlined.
1443	5'-GCTAGTCTAGATTACTTGTACAGCTCGTCCATGCCG- 3'; Reverse primer to amplify mCherry for constructing pSSK45; XbaI site underlined.
1463	5'-AACAGTATCGATGTTTCGCATTTAG-3'; Used for confirming and sequencing Fjoh_4175 deletion.
1464	5'-GCAAAGAGCGCCAAGTTTAC-3'; Used for confirming and sequencing Fjoh_4175 deletion.
1516	5'-GCTAGGGATCCCCTACTTTTTTCCCGTGGGCTGGCTG -3'; Reverse primer to amplify short N-terminal region of <i>chiA</i> to construct pSSK52 and pSSK54; BamHI site underlined.
1593	5'-GCTAGGGTACCTCCCCGGTAGAGATAGTTATGGCTAT -3' Forward primer to amplify N-terminal region of <i>chiA</i> to make constructs pSSK52,,and pSSK54; Binds 400 bp upstream of <i>chiA</i> start codon; KpnI site underlined.
1600	5'-GCTAGTCTAGAGCTTATGCAGCTTATTTTCGCATCACAA -3' forward primer to amplify <i>chiA</i> CTD region for making construct pSSK52; XbaI site underlined