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"

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   1 MKHYYRLLFLLLFPLLASAQPAHGKKVVGYYAQWSIYARDFNVPKIDGSK
  51 LTHLNYSFYGTTYDPAHPENTKLKCLDTYADFEHMEGGIPWDAPVKGNFY
 101 DLMKLKQKYPHLKILISVGGWTKGQDLSPIAASPVARAALAADMANFIVT
 151 YPFIDGFDIDWEYPLSGGTDGTEIVNGMPVPPQKYSPDDNKNLVLLLKAM
 201 RQAMPNKLVTIAAGNNVRNVSKQYLGPNNRAQYGMTEDISTYCDYITYFG
 251 YDFGGNWYDKTCYNAPLYASGNPNDPLYGATQSESLDELTNQYLNVIGFP
 301 ANKLIMGLPFYGKKFDNVAANSTNGLFVAAPRYIVPGCTNPQNPTGTWDG
 351 SGACEKSGSIEICDLVGNPVTNSHAYLDPNTMMVTPSAASAGWVRYFDNT
 401 TKVPYLYNSTLKQFISYEDKQSMDLKVQYIKSRNLAGGMIWELSQDTRGS
 451 IPNSLLNQVDTSFGSVVPGTVSISGSVKNGSALVTDVTVELRNASNAVIQ
 501 TVVSANGNFAFNNLTSGQNYSLTALKATYTFTPVTLVNVTVNQTAVVING
 551 TQPTYTVSGTVLDGSTPVSGVTVTAVSGSTTLTAVSNASGVYSIAGLTAG
 601 LNFTVTAAKSGFSYAPASTVYNAIDSNKTLNFTQGAPVVNYTVSGTVLNS
 651 TTPVSGVTVTASFTGGSYAAVTNASGTYSLSLPSGGNYTVTAALTGQTFT
 701 PASTVYSNLNANKTLNFTQDVVVSTSKISGTVKNGTNPVAGAKVELVLPW
 751 TDNTHNWKSVIATTDAQGKYSFDNSVVDGYTQVLSLKLNSWQNGEVAYYP
 801 NNLANFAVPANPTVYNFNTSSTAKSALAAAANLISGTVKNGTTPVAGAKV
 851 EIVLPWTDNTHNWKSVLATTDASGNYSFDNSVVAGYTQILSLKLNGWENG
 901 DVTYYPNNLANFAVPTTPTIYNFNRQAVVATKPVVTITAPTASAIAINLG
 951 SAINFVASVGLSAVDATTISSVVFSLDGQSLSTANSSGTYTAAWTPAANQ
1001 FSLSHTLTVTATASNGTTDSKTYSFTLTCSGANCPNALPVITWNSPSNTT
1051 VYQNTFQVVPISVTAVDSDGTVSGVTITINGGTFNMTAGTNNTYTYNFTP
1101 SAYQDYPVVIKATDNKSGVTTLNNTIKIATVSTNRFIPLPSKIILGYAHS
1151 WENAGAPFLYFSQMVGSKFNVVDYSFVETVNRDGYTPILTTNDTRYLTNG
1201 VFNKQLLKNDIKSLRDSGVPVIVSIGGQNGHVVLDNVTQKNIFVNGLKAI
1251 IDEYQFDGVDIDFEGGSMNFNAGGLRDISYAGISAYPRLKNVVDAFKELK
1301 AYYGPGFLLTAAPETQYVQGGYTTYTDTFGSFLPIIQNLRNELDLLAVQL
1351 YNTGGENGLDGQYYGTAKKSNMVTALTDMVIKGYNIASTGMRFDGLPASK
1401 VLIALPACPSAAGSGYLTPTEGINAMHYLRTGTTFSGRTYTMQPGGPYPS
1451 LRGLMTWSVNWDASSCGNSSELSKAYAAYFASQTAAKTLVLDDISAKSNA
1501 TIAYFKNNALSVTNENEDIAQVDVFNVLGQNLVSHRNVQNNKEVLLHNQS
1551 FSSKQLFLVVVTDKAGNKKSFKVMNFLN
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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.

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.

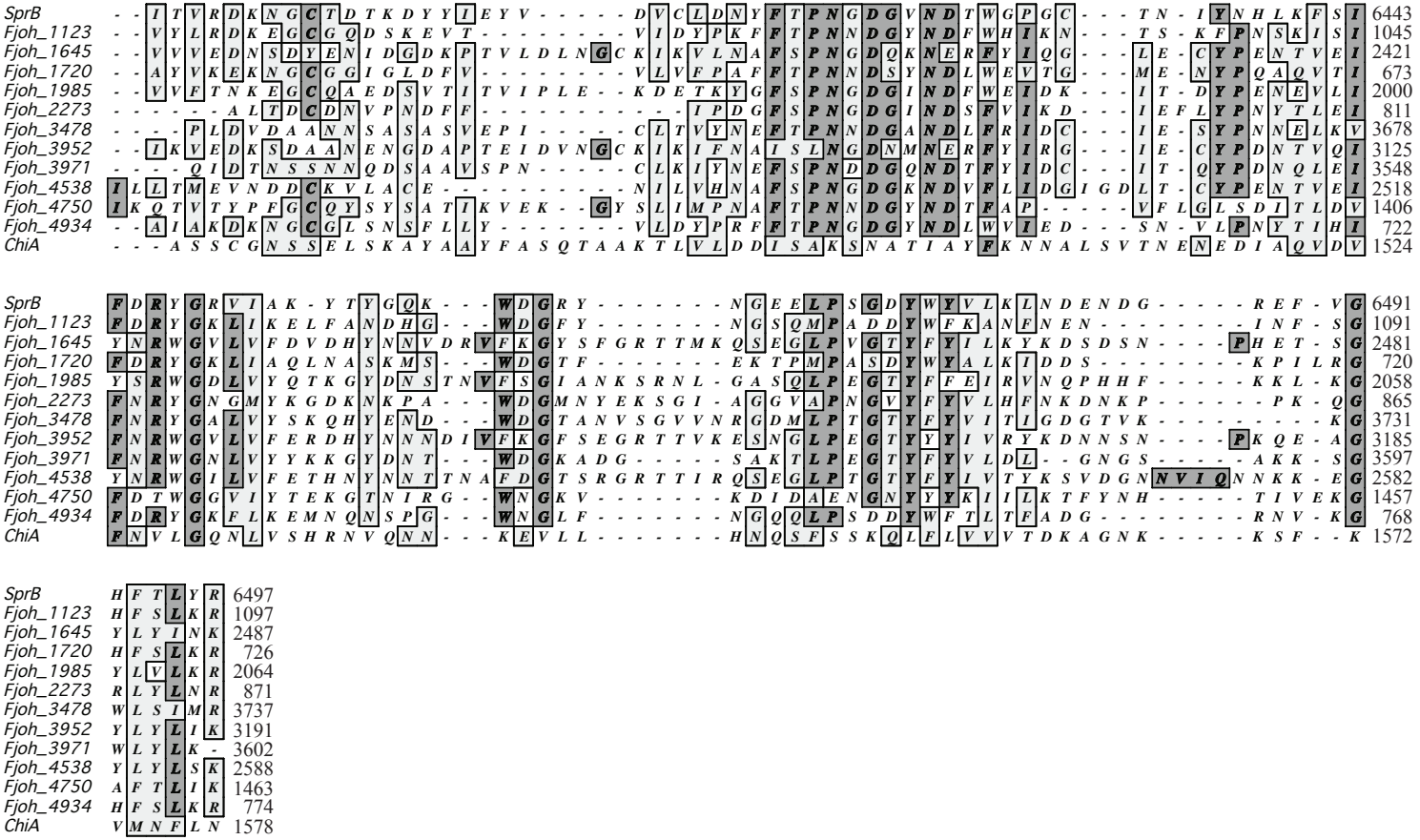
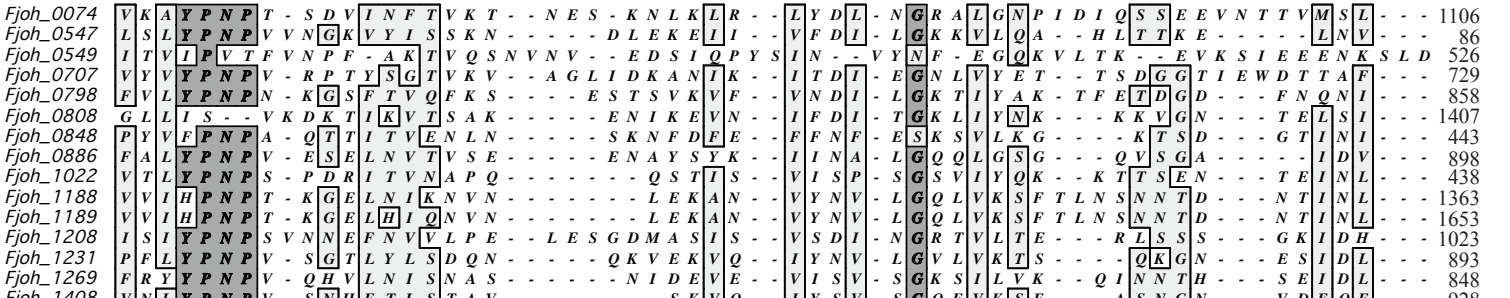


Figure S3. Alignment of the C-terminal domain (CTD) of ChiA with the CTDs of *F. johnsoniae* TIGR04131 family members using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. TIGR04131 is described as 'gliding motility-associated C-terminal domain' on 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



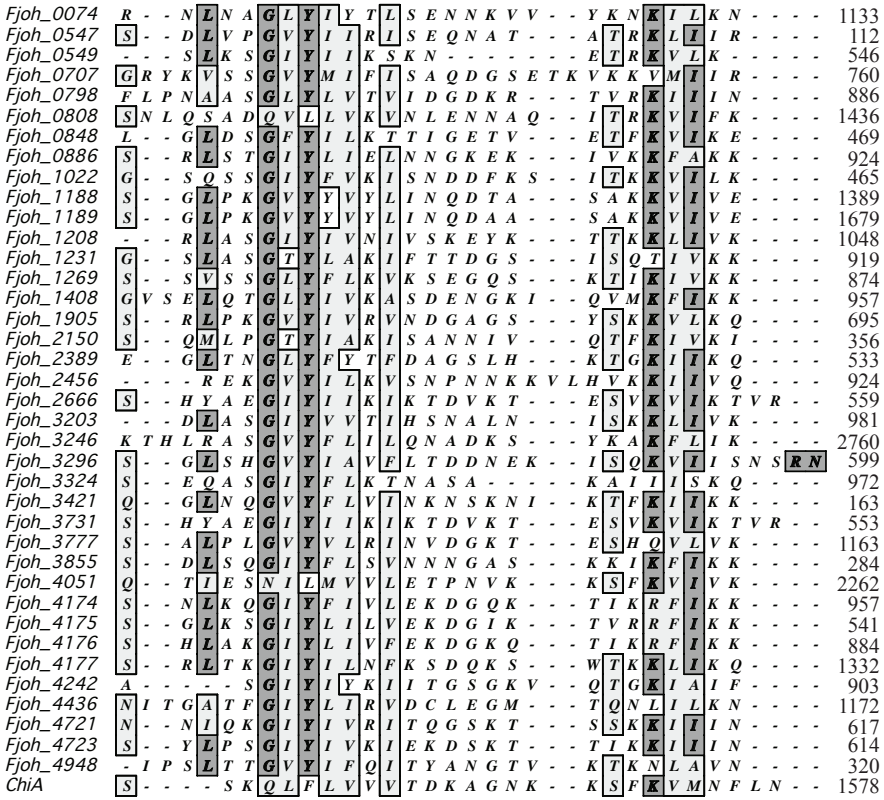


Figure S4. Alignment of the C-terminal domain (CTD) of ChiA with the CTDs of *F. johnsoniae* TIGR04183 family members using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. TIGR04183 is described as 'Por secretion system C-terminal sorting domain' 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.

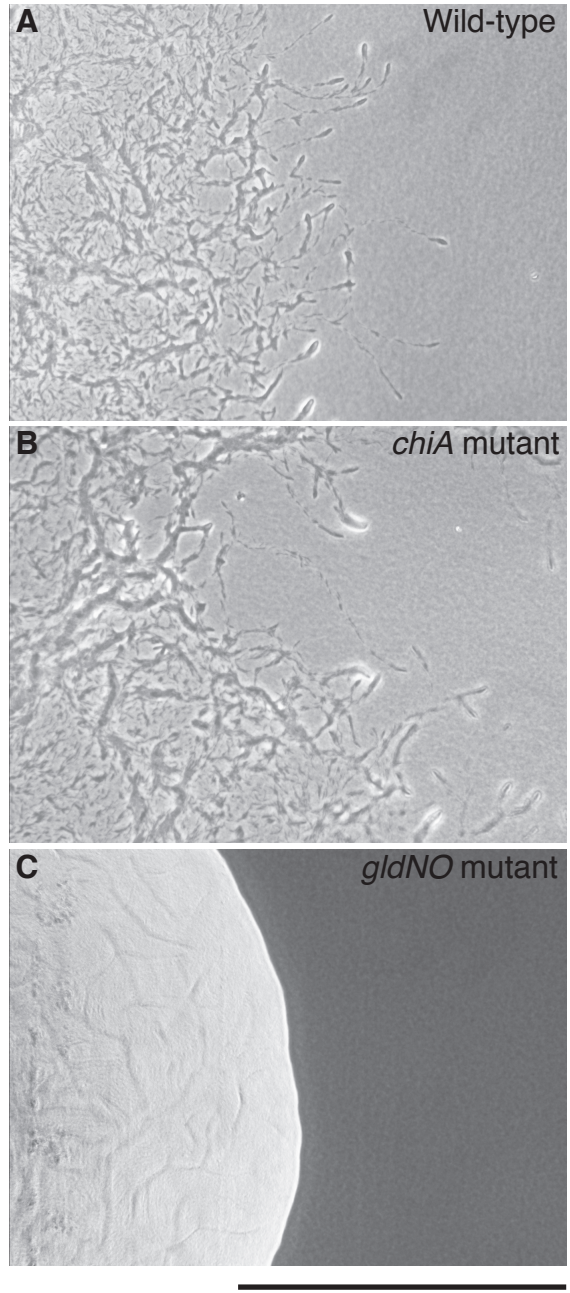


Figure S5. Disruption of *chiA* does not affect gliding motility. Colonies were grown for 42 h at 25°C on PY2 agar medium. Photomicrographs were taken with a Photometrics CoolSNAP$_{cf}^{2}$ camera mounted on an Olympus IMT-2 phase-contrast microscope. (A) Wild-type *F. johnsoniae* UW101. (B) *chiA*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

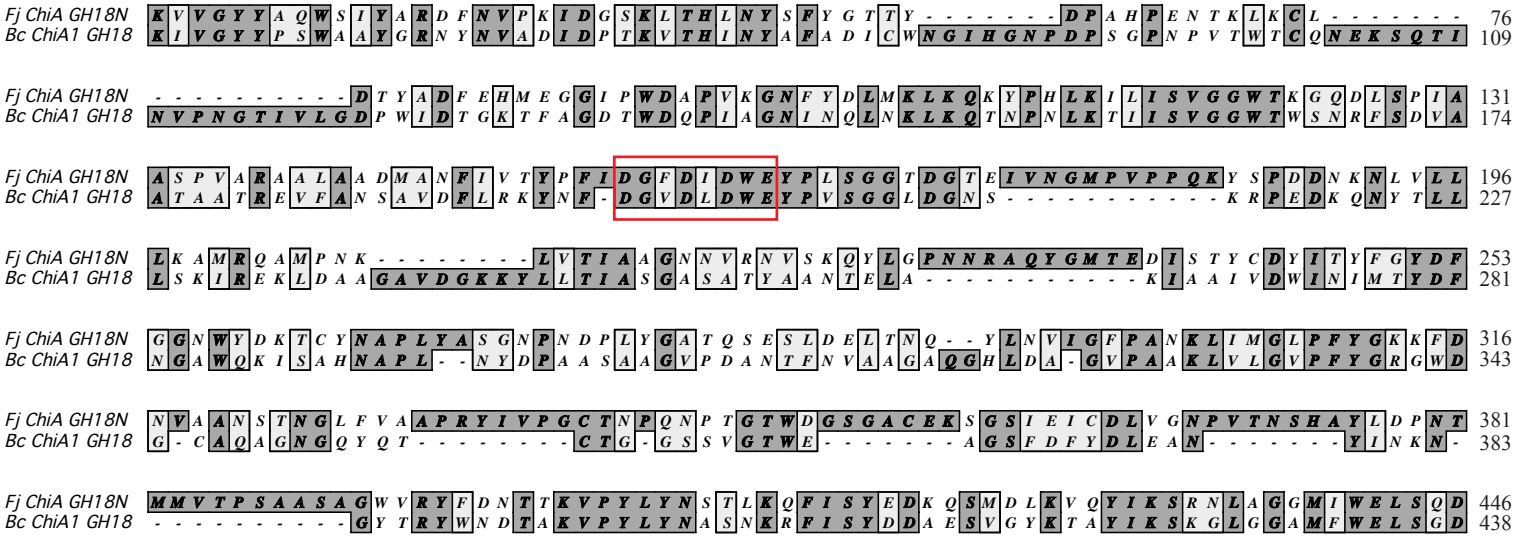


Figure S6. Alignment of *F. johnsoniae* $ChiA_{GH18N}$ with the GH18 domain of *Bacillus circulans* ChiA1 using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. The conserved 'DXXDXDXE' GH18 catalytic site is boxed in red.

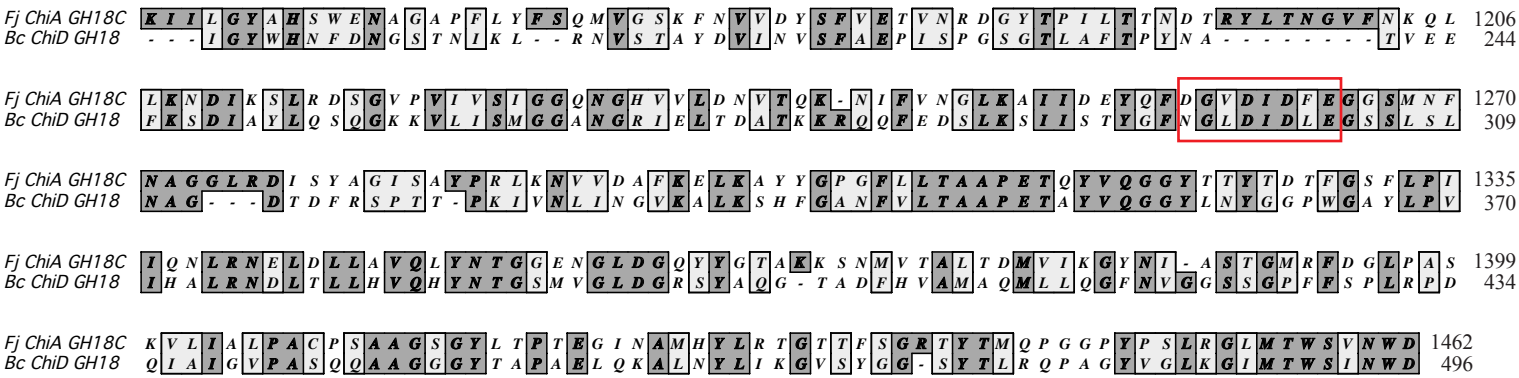


Figure S7. Alignment of *F. johnsoniae* $ChiA_{GH18C}$ with the GH18 domain of *Bacillus circulans* ChiD using MUSCLE. Dark shading indicates identical amino acids and light shading indicates similar amino acids. The conserved 'DXXDXDXE' GH18 catalytic site is boxed in red.

Table S1. Primers used in this study.

Primers	Sequence and Description
737	5'-AGGCACCCCAGGCTTTACACT-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; SalI site underlined.
941	5'-TTGCACCTGCAACCGGATTTGTTC-3'; Reverse primer used for confirming and sequencing *chiA* disruption mutant CJ1808; Binds 532 bp upstream of primer 937.
974	5'-GCTAGTCTAGAGGTTCATAATGCGCATCCTTAGGCA-3'; Reverse primer used to amplify *chiA* for construction of complementation plasmid pSSK05; XbaI site underlined.
975	5'-GCTAGGGATCCCTTCCAACCTGCAGTTGAGCGAAA-3'; Forward primer used to amplify *chiA* for construction of complementation plasmid pSSK05; BamHI site underlined.
1066	5'-GCTAGGGATCCAGTCCGGTAGCAAGAGCTGCATTA-3'; Forward primer used to amplify *chiA* for constructing pSSK07; BamHI site underlined.
1067	5'-GCTAGGTCGACTTTTGCACCTGCAACCGGATTTGTTC-3'; Reverse primer used to amplify *chiA* for constructing pSSK07; SalI 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; SalI site underlined.
1229	5'-GCTAGGTCGACTTCTTCTCGGCAGAAGTTTCGGGA- 3'; Forward primer used to amplify downstream region of Fjoh_4175 for constructing pSSK32; SalI 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 *chiA* CTD for constructing pSSK26; BamHI site underlined.
1379	5'- GCTAGGTCGACAGATAATTCAGATGAATTACCGCAAGA-3'; Reverse primer to amplify the upstream region of *chiA* CTD for constructing pSSK26; SalI site underlined.
1380	5'-GCTAGGTCGACAACTAATAAATGATTGAAAATTTAGAA -3'; Forward primer to amplify the region downstream of *chiA* for constructing pSSK27; SalI site underlined.
1381	5'-GCTAGGCATGCTGAAATTTCCATTAGCCAGC -3'; Reverse primer to amplify the region downstream of *chiA* for constructing pSSK27; SphI site underlined.
1391	5'-TCTGGAAGAACATATACTATGCAGCCA- 3'; Forward primer used to confirm and sequence *chiA* CTD deletion.
1392	5'-TCACCTAATACAATAACTAACCTC-3'; Reverse primer used to confirm and sequence *chiA* CTD deletion.
1404	5'-GCTAGGCATGCTCACCTAATACAATAACTAACCTC-3'; Reverse primer to amplify *chiA* 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'-GCTAGGGATCCCACTACTTTTTTCCCGTGGGCTGGCTG -3'; Reverse primer to amplify short N-terminal region of *chiA* to construct pSSK52 and pSSK54; BamHI site underlined.
1593	5'- GCTAGGGTACCTTCCCCGGTAGAGATAGTTATGGCTAT -3' Forward primer to amplify N-terminal region of *chiA* to make constructs pSSK52,,and pSSK54; Binds 400 bp upstream of *chiA* start codon; KpnI site underlined.
1600	5'GCTAGTCTAGAGCTTATGCAGCTTATTTCGCATCACAA -3' forward primer to amplify *chiA* CTD region for making construct pSSK52; XbaI site underlined