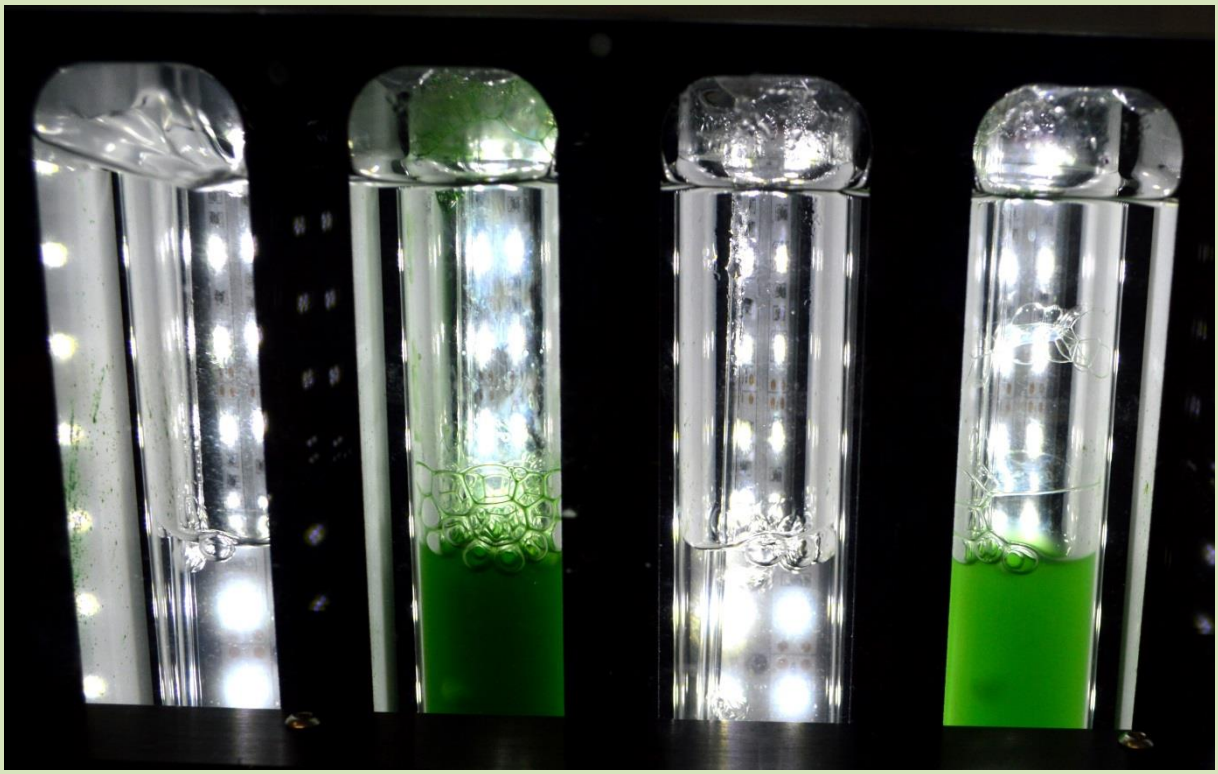
M.Res. Molecular Plant and Microbial Sciences, Rotation 2 Project Report, August 2017

Phenotypic and Proteomics-Based Evidence for the Schizokinen Siderophore Synthesis Operon in the Cyanobacterium *Anabaena* sp. PCC 7120

Peter Wellham

Imperial College London



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Contents

Abstract

Iron is an essential element in the metabolism of many bacteria, and thus specialised mechanisms for its uptake from iron-limited environments have been described in many species. Here the ferric iron-chelating siderophore schizokinen, of the cyanobacterium *Anabaena* sp. PCC 7120 was investigated, focussing on the proposed but not fully characterised operon (*All0390-All0396*) for its biosynthesis. Wild type, full operon knock-out, full complementation, and *All0396* and *All0395* single-gene knock-out strains were the subject of phenotypic and proteomic experiments. Following growth assays conducted under varying iron-availability conditions and proteomic analyses performed via liquid chromatography–mass spectrometry, strong evidence emerged for the role of this set of genes as well as the importance of schizokinen in cell growth, particularly concerning the performance of this cyanobacterium under iron-limited conditions rendered by the presence of the chelator 2,2'-dipyridyl, and by an absence of ferric iron.

Introduction

Anabaena sp. PCC 7120 and the Issue of Iron Accessibility

Cyanobacteria, often referred to as the "blue-green algae", are photosynthetic gram-negative bacteria, perhaps most well known in industry for their importance in the nitrogen cycle of soil environments [21]. Some species of cyanobacteria, including members of the genus *Anabaena*, are able to anaerobically fix nitrogen, which takes place in heterocysts [10] – larger, specialised cells which can differentiate from a small proportion of vegetative cells which form colonial chains [3]. With a focus on anaerobic nitrogen-fixing capabilities and the potential further applications in agricultural settings such as paddy fields, cyanobacteria have become a research organism of interest. Several numbered subspecies of *Anabaena*, such as PCC 7120 [1], the genome of which has been sequenced [8], have been maintained in laboratories since their collection from freshwater [21]. *Anabaena* sp. PCC 7120, studied in this project, has retained the ability to fix nitrogen [4]. Here the importance of another facilitating aspect of metabolism is investigated – a key iron uptake strategy deployed by this cyanobacterium: the use of endogenous iron-chelating siderophores.

For the first billion years of life on Earth, anaerobic conditions ensured the abundance of iron accessible for use in the centres of ancient proteins, particularly Fe-S clusters, by prokaryotes [5]. 2.75 billion years ago, following the evolution of photosystem II in cyanobacteria, oxygenation of the atmosphere began. The presence of oxygen causes the formation of unstable compounds from Fe-S proteins, and thus anaerobes had to develop new strategies of gaining iron, or simply retreating to anaerobic sanctuary. Subsequently anaerobes are vulnerable to oxidative stress and reduced iron availability, as Fe-S cluster proteins are in many cases irreplaceable as redox enzymes, and in the case of cyanobacteria, for example, are essential in photosynthesis [5].

In numerous cases, bacteria are known to have evolved siderophores, strong chelators of e.g. iron which form highly stable complex ions [31]. In gram-negative bacteria including the cyanobacteria, the evolution of iron siderophores was simultaneous with that of the TonB system, which couples a proton motive force to transporters on the outer membrane, via proton-pump-charged plasma membrane-located TonB – a solution to the problem of siderophore-chelates being too large to fit through porins [28; 32]. However, siderophores are metabolically expensive due to their single-use property – this is because once inside the cell, only hydrolysis of siderophores can release iron. In cyanobacteria, the Fur (ferric uptake regulation) protein system acts to supress and regulate the expression of the biosynthesis of siderophores and their uptake genes; thus these genes have greater expression under iron-limiting conditions [17]. Fur proteins are also present in many other bacteria as master regulators of iron metabolism, use iron ions as corepressors and bind to conserved Fur box/iron box sequences in the promoter regions of relevant genes [9].

Schizokinen, an Iron-Chelating Siderophore

One iron-chelating siderophore released by *Anabaena* 7120 is schizokinen [11], which is transported back into the cell after chelation of ferric iron by a transporter proteins SchT (locus *Alr0397*) [18], and IutA2 (locus *Alr2851*) [23]. These are two examples of TonB-dependent transporters (TBDTs) – β-barrell proteins located in the outer membrane powered by proton-motive forced provided via TonB in the plasma membrane [20]. The schizokinen transport system is also proposed to rely on additional protein factors located in both the plasma membrane as integral proteins and in the periplasmic space, including those encoded by *Exb* and *Fhu/Fut/Fec* family genes as well as TonB [8; 16; 27] and a TolC-dependent exporter [19]. Other siderophores in *Anabaena* 7120 have also been detected, for example genes for synthesis of another siderophore at loci *All2643-50* have been described [6]. In a previous study, *Alr0397* knock-out mutants showed a phenotype of iron-starvation under iron-limited conditions, and under those conditions, wild-type *Anabaena* 7120 showed highly elevated expression levels of the schizokinen transporter [18], so it is hypothesised that schizokinen is particularly important for iron uptake by *Anabaena* 7120 and that its use is regulated via the iron homeostasis of the cell. Furthermore, the *Alr0397* locus is proximate to a FurA (ferric uptake regulator A) binding site, near to several candidate genes for the biosynthesis of schizokinen from aspartate semi-aldehyde [15], although this pathway has not yet been metabolically characterised (see appendix figure C(ii)). The candidate loci for the biosynthesis of schizokinen – *All0390, All0392, All0393, All0394, All0395,* and *All0396* – are hypothesised to form an operon with the transporter gene *Alr0397* (see appendix figure C(i) and appendix C) due to sequence similarities to siderophore biosynthesis gene clusters in *Anabaena variabilis, Geitlerinema* sp. PCC 7407, *Cyanobacterium* sp. PCC 10605, *Synechococcus* sp. PCC 7002, *Leptolyngbya* sp. PCC 7376, and *Sinorhizobium meliloti* 1021 [15]. The putative biosynthesis pathway is proposed to be similar to that of the *S. meliloti* siderophore rhizobactin 1021, starting with L-aspartate semialdehyde [15] (see appendix figure C(ii)). Like the schizokinen transport system, rhizobactin transport in *S. meliloti* is powered by factors including TonB and ExbB-ExbD, involing proton-motive force [29].

This project combines phenotypic and proteomic experiments to assess the importance of the proposed schizokinen operon under iron conditions and provide evidence of its role in the synthesis of an effective iron-chelating siderophore. Growth assays of various operon mutant strains and the wild type were conducted in liquid BG-11 media types with varying iron contents and accessibilities – involving the use of the chelator 2,2'-dipyridyl. The mutant strains were created by a previous student at the Microbial Metabolic Engineering (Jones) Lab, and consisted of a double-recombinant *All0390* – *All0396* and *Alr0397* knock-out mutant, a fully-complemented version of the knock-out mutant via replacement of the genes on a plasmid, and other two different complemented mutants with similar plasmids but lacking the genes *All0396* and *All0395* (see table 1 and appendix C). As well as growth assays, further phenotypic tests involved the use of the Chrome Azurol S (CAS) assay for the detection of siderophores [24] adapted for *Anabaena* [18]. The various mutants and the wild type were also the subject of proteomic tests using liquid chromatography–mass spectrometry targeting proteins of interest including those involved in the proposed synthesis pathway of schizokinen, following culturing in different iron accessibility conditions.

Materials and Methods

Phenotypic tests on growth performances of the various schizokinen operon mutants – referred to as *Anabaena* schizokinen mutant strains growth assays – combined with proteomic testing via mass spectrometry form the main experiments of this study.

Phenotypic Evidence: *Anabaena* Schizokinen Mutant Strains Growth Assays

Growth assays, each with a duration of two weeks, were performed on different (proposed schizokinen operon) mutant strains of *Anabaena* 7120 as well as the wild type, in BG-11 media types containing varying availabilities of iron – as detailed in tables 1, 2, and 3. In all cases, contamination was checked at the start and end of assays by streaking on LB agar, and sterile conditions were maintained throughout.

Assembling and Confirming Mutant Strains

Mutant strains previously created by a former student at the Microbial Metabolic Engineering (Jones) Lab [15] were revived from frozen stocks (preserved at -80°C with 10 per cent glycerol) by streaking onto selective BG-11 solid agar media and culturing at conditions of elevated (1 per cent) carbon dioxide, 30°C and a light intensity of 60 µE for 2-3 weeks. In each case, when setting up growth assays using the mutant strains, BG-11 liquid media cultures containing the relevant antibiotic (see table 1) were prepared 2-3 weeks in advance using cells from the BG-11 agar plates. Initially, the identities of mutant strains revived from glycerol stocks were confirmed by colony PCR (see appendix A).

A total of five strains, including wild-type *Anabaena* sp. PCC 7120 [1; 21] and four mutant strains of this subspecies were tested in the *Anabaena* Schizokinen mutant strain growth assays. Assembling mutant strains involved double recombination using a plasmid containing relevant operon 5’ and 3’ flanking sites and to create a whole-operon (*All0390-All0396*, not including the transporter gene *Alr0397*) knock-out strain, followed by various degrees of complementation via the introduction of other plasmids, containing the operon, to create complementation strains and single-gene mutants. Antibiotic resistance marker genes were also involved to enable selection of mutant strains. The details of these are documented in table 1 and relevant plasmid and genomic operon DNA sequences in appendix C.

Table 1: Mutant Strains and their Markers*

Strain	Explanation	Antibiotic Resistance Markers
WT (Wild Type)	-	-
KO (Knock Out)	Double recombinant knock out of the operon, genes *all0390*, *all0391*, *all0392*, *all0393*, *all0394*, *all0395*, *all0396*, and *alr0397*	Spectinomycin, Streptomycin
FC (Full Complement)	Double recombinant knock out as above, complemented fully for genes *all0390* – *all0396* and *alr0397* via RSF1010 plasmid	Neomycin, Spectinomycin, Streptomycin
FC – *all0396*	Double recombinant, complemented via RSF1010 plasmid, for genes *all0390* – *all0395* and *alr0397* (not *all396*)	Neomycin, Spectinomycin, Streptomycin
FC – *all0395*	Double recombinant, complemented via RSF1010 plasmid, for genes *all0390* – *all0394, all0396* and *alr0397* (not *all0395*)	Neomycin, Spectinomycin, Streptomycin

* See appendix C for more details

Different Media Types

Blue-Green media 11 (BG-11, specifically BG-11_0 media – a variant, lacking a nitrate source [21; 25; 26] was used as the foundation media throughout all growth assays. Four media types were prepared, two of which (types 2 and 4) contained the iron chelator 2,2'-dipyridyl, at a final concentration of 50 µM, which has been found previously to cause phenotypically-observable iron-deprived for *Anabaena* 7120 [12]. Media types 3 and 4 lacked ferric ammonium citrate, unlike standard BG-11 in the other types in which 0.006g/l (22.9µM) was present, that being the only iron source.

Table 2: Media Types Used in the *Anabaena* Schizokinen Mutant Strains Growth Assays

Media ID	Ammonium Ferric Citrate Presence	Iron Content	Chelator (2,2'-Dipyridyl) Presence
1	Yes	22.9 µM ($C_6H_8FeNO_7$)	No
2	Yes	22.9 µM ($C_6H_8FeNO_7$)	Yes
3	No	0	No
4	No	0	Yes

Table 3: Treatment Groups of the *Anabaena* Schizokinen Mutant Strains Growth Assays – Conducted in 6-Well Plates, and the Multi-Cultivator 1000

Apparatus used	Carbon Dioxide Concentration	Strain*	Media ID**	Sample size (Replicates) ***	Total Number of Treatment Groups	Total Number of Datasets
6-well plates	Elevated (1%)	WT	1	3	20	60
		KO		3		
		FC		3		
		FC – *all0396*		3		
		FC – *all0395*		3		
		WT	2	3		
		KO		3		
		FC		3		
		FC – *all0396*		3		
		FC – *all0395*		3		
		WT	3	3		
		KO		3		
		FC		3		
		FC – *all0396*		3		
		FC – *all0395*		3		
		WT	4	3		
		KO		3		
		FC		3		
		FC – *all0396*		3		
		FC – *all0395*		3		
MC1000	Atmospheric	WT	1	6	8	45
		KO		5		
		WT	2	6		
		KO		6		
		WT	3	6		
		KO		4		
		WT	4	6		
		KO		6		

* Properties of mutant strains are detailed in table 1.
** Media types corresponding to ID numbers are detailed in table 2.
*** Different numbers of replicates have occurred in the MC1000 treatment groups due to contamination issues, and the exclusion of affected datasets.

Growth Assays in 6-Well Plates

All five strains were tested with all four media types (see tables 1 and 2), with therefore a total of 20 treatment groups (see table 3), with three replicates for each, in 3 ml 6-well plate-based growth assays. From selective flask precultures (see above), cells were washed three times in BG-11 media by resuspension and pelleting to remove residual media and antibiotics. Optical density measurements at 720 nm were taken daily, using 10X dilutions of culture samples measured in 96-well plates as described for the preliminary assays, for a total duration of two weeks. Cultures were kept at 30°C, 1 per cent carbon dioxide, and at 60 µE light intensity.

Growth Assays in the Multi-Cultivator 1000

Further growth assay data was collected for the wild type and knock-out strains in all four media types (therefore eight treatment groups), using the MC1000 incubator (figure 1). Cells were washed in the same way as prior the 6-well plate assays, and likewise a starting optical density at 720 nm of 0.02 (as measured by an Infinite® 200 PRO NanoQuant Multimode Microplate Reader (Tecan)) was set for the 50 ml cultures. Measurements of optical density at 720 nm were taken every 30 minutes for 14 days. Cultures were kept at 30°C, atmospheric carbon dioxide, and at 60 µE light intensity.

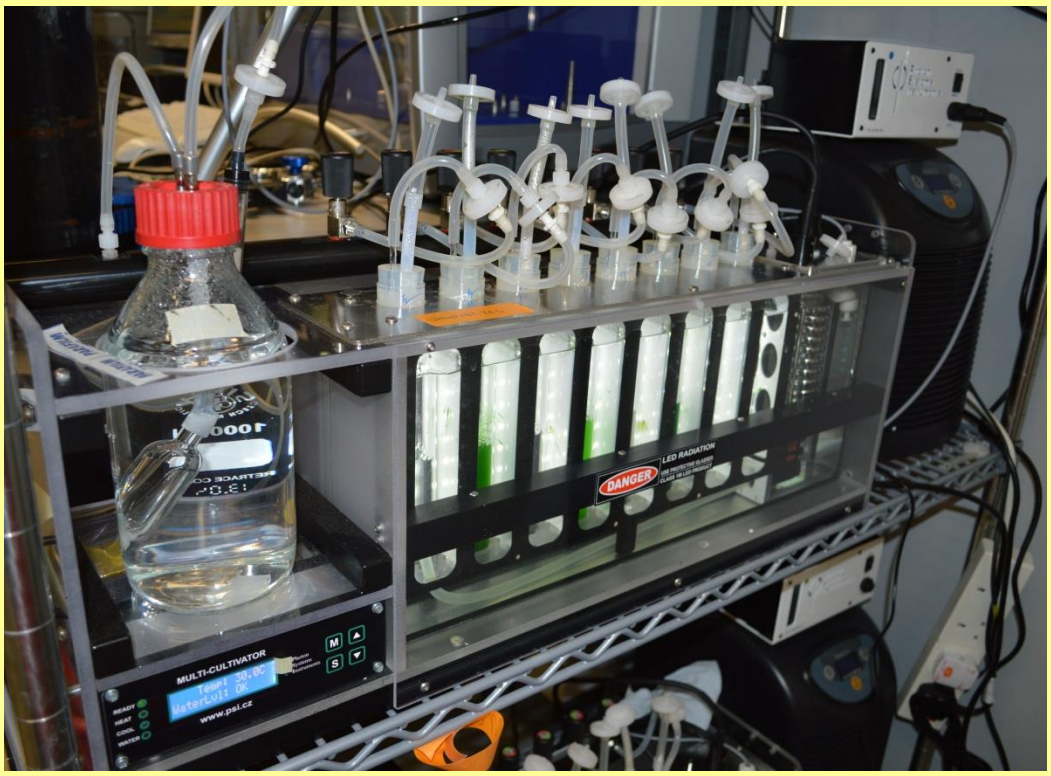


Figure 1: Multi-Cultivator 1000 Set Up During a Growth Assay.

Statistical Analysis of Growth Assay Data

Statistical analyses of growth assay data were performed with in R (3.0.2) using the Grofit package (https://cran.r-project.org/web/packages/grofit/grofit.pdf), to fit growth curve models and estimate informative parameters. In each case, datasets were tested on their ability to fit certain models – logistic, gompertz, richards, and modified gompertz (gompertz exponential) – and hence suitable models were chosen to compare datasets [7]. Growth curves from raw values, and also from mean values of treatment groups were assessed.

Phenotypic Evidence: Chrome Azurol S Chelator Assay

Chrome Azurol S (CAS) assays [24] were performed in a suitable manner for the detection of siderophores released by *Anabaena* 7120, according to the protocol used by Nicolaisen *et al.* 2008 [18] (see appendix A). This assay relies on CAS and HDTMA (hexadecyltrimethylammonium bromide) complexing with ferric iron causing a blue colour in the media. CAS and HDTMA act as indicators for iron, such that a blue to yellow colour change can be observed when a siderophore, acting as a strong chelator, binds to and effectively removes iron from the media [13]. Colonies from BG-11 plates of all mutant strains and the wild type were streaked onto CAS plates, and photographed 14 days later.

Proteomic Evidence: Mass Spectrometry of Proteins

In order to gain insight into levels of expression of several genes of interest in each strain under different iron conditions, proteomic tests were conducted, in the form of liquid chromatography-mass spectrometry following protein extraction and trypsin digestion. These were undertaken from dense (OD_{720} >1) starting cultures; and protocols used are described in appendix A. Ten treatment groups were considered – each of the four mutant strains as well as the wild type (see table 1 and appendix C), treated to different growing conditions – standard BG-11 (media type 1 in table 2) and BG-11 without ferric ammonium citrate (media type 3). Cells in the given treatment groups for the latter media were treated to three days' exposure to the media after removal from media type 1 – this being due to the insufficiently slow growth of precultures in media type 3. Digested protein samples were introduced into the liquid chromatography-mass spectrometer (see appendix A for more detailed protocols). Proteins of interest were selected for an initial transition list which was assembled by *in silico* trypsin digest predictions using Skyline® (MacCoss Lab Software) and this was later refined for subsequent runs of the mass spectrometer with scheduled retention times (see appendix E). MRM (multiple reaction monitoring) and EPI (enhanced product ion) data was analysed using Analyst® software (SCIEX). Proteins encoded by proposed schizokinen synthesis operon genes *All0390* – *All0396* as well as the schizokinen transporter gene *Alr0397*, proposed siderophore transporter gene *All2650* [6], Photosystem II Chlorophyll A–binding protein–encoding gene *All4001* (*isiA*), an oxidative stress-response protein known to have elevated expression in *Anabaena* 7120 under iron stress [34], were selected as proteins of interest, as well as the large subunit of ribulose bisphosphate carboxylase (RuBisCO) – a positive control. (See appendix E for protein sequences and transition lists.)

Results

Prior to starting growth assays and other experiments on schizokinen operon mutant and wild type strains, the identities of strains were successfully confirmed by colony polymerase chain reactions using relevant primers, as shown in figure 2, tables 4 and 5, with full details presented in appendix C. From this point onwards, appropriate antibiotic pressures were maintained in all liquid cultures (see table 1) – to ensure that properties of different strains were not lost and to prevent contamination.

Table 4: Primers Used in Colony PCRs (figure 2; see appendix C)**.**

Primer	Sequence (5' – 3')	Primer Binding Location
62	GCCTAAGATGTTTCGCAACTCC	*all0394*
124	TATGGCTAGTGACACAATCC	*alr0397*
125	GTCCATAACCAATCAATTCCC	3' Flank
149	ATACCATGCTCAGAAAAGGC	RSF1010 Backbone
175	GGACACACAGCGATATCTGC	*all0395*
176	TTTGATTGTGACTCCAGCGC	*all0396*

Table 5: Colony PCRs – Strains Tested and Primers Used (figure 2; see appendix C)**.**

Lane	Strain (Template DNA)	Primers	Expected	Gel	Purpose
2 (Fig. 2a)	WT	176+62	3505	✓	Proves presence of operon
3 (Fig. 2a)	KO	124+125	2046	✓	Proves genomic operon KO
4 (Fig. 2a)	FC	124+125	2046	✓	
5 (Fig. 2a)	FC –*all0396*	124+125	2046	✓	
6 (Fig. 2a)	FC –*all0395*	124+125	2046	✓	
7 (Fig. 2a)	FC	149+62	5469	✓	Proves presence of RSF1010 plasmid
8 (Fig. 2a)	FC –*all0396*	149+62	3988	✓	
10 (Fig. 2a)	FC –*all0396*	149+175	860	✓	Proves *all0396* missing from plasmid
11 (Fig. 2a)	FC –*all0395*	176+62	1889	✓	Proves *all0395* missing from plasmid
2 (Fig. 2b)	FC –*all0395*	149+62	3853	✓	Proves presence of RSF1010 plasmid

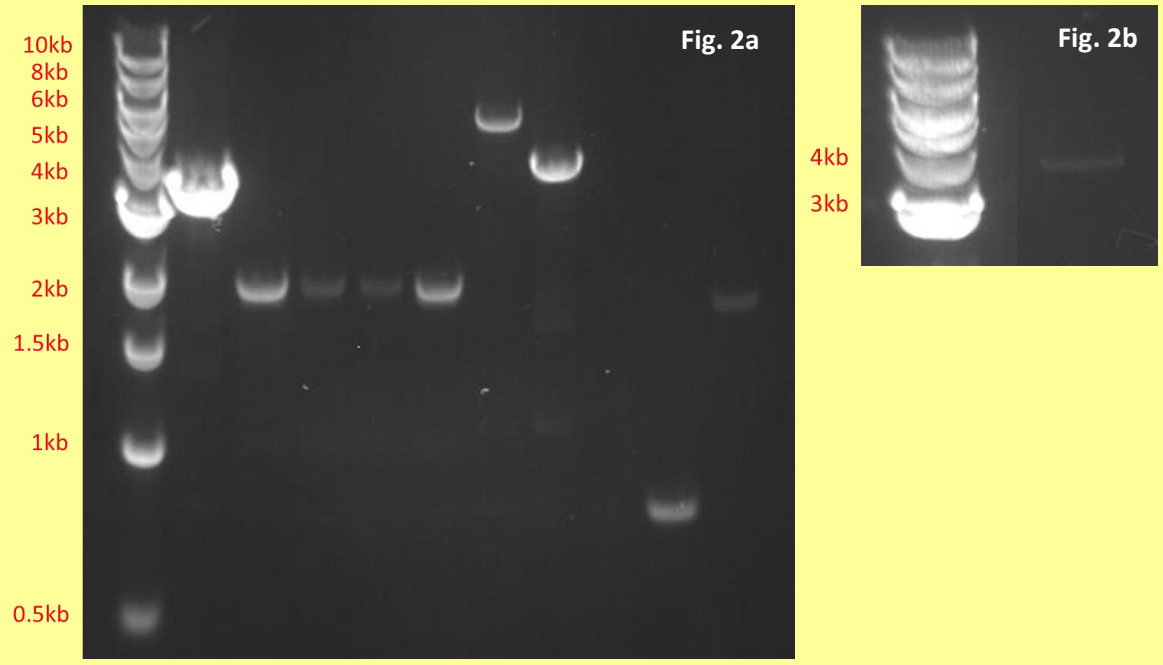


Figure 2: Colony PCRs Confirming Schizokinen Operon Mutant Strains and the Wild Type. Ladder used in both gels: 1kb Ladder (New England Biolabs). Primers used and strains tested are detailed in tables 4 and 5.

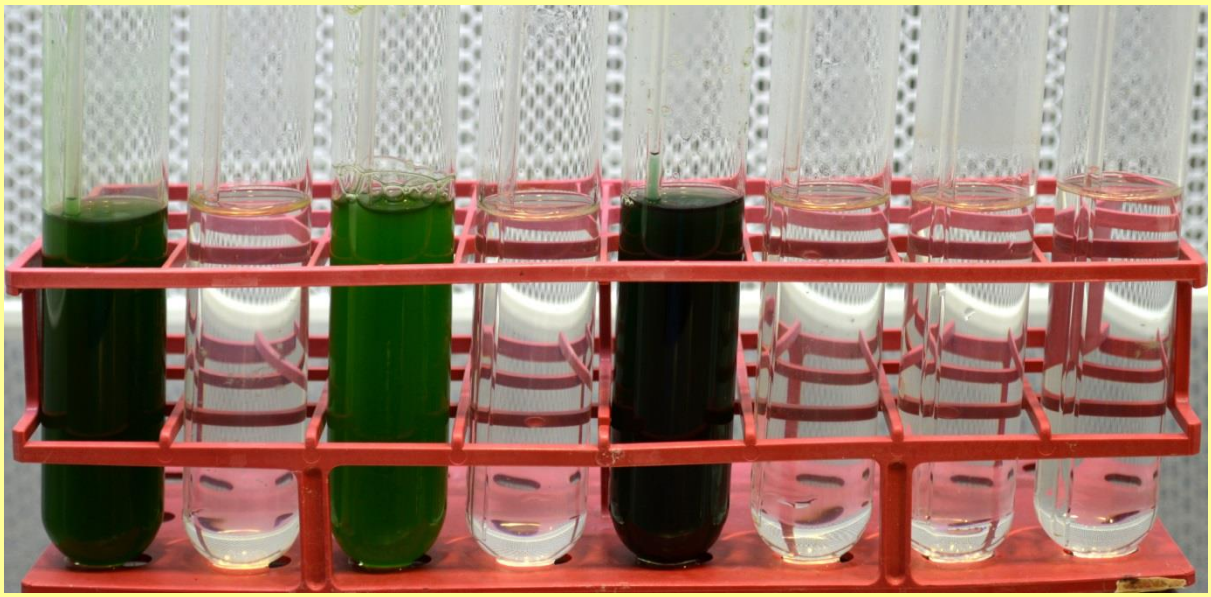


Figure 3: Photograph of Multi-Cultivator 1000 Tubes Containing Growth Assays of the Wild Type and Knock-Out Strains with Different Media Types (*Anabaena* Schizokinen Mutant Strains Growth Assays). Tubes from left to right – strain, media type: WT,1; WT,2; WT,3; WT,4; KO,1; KO,2; KO,3; KO,4 (see tables 1 and 2).

Figure 4

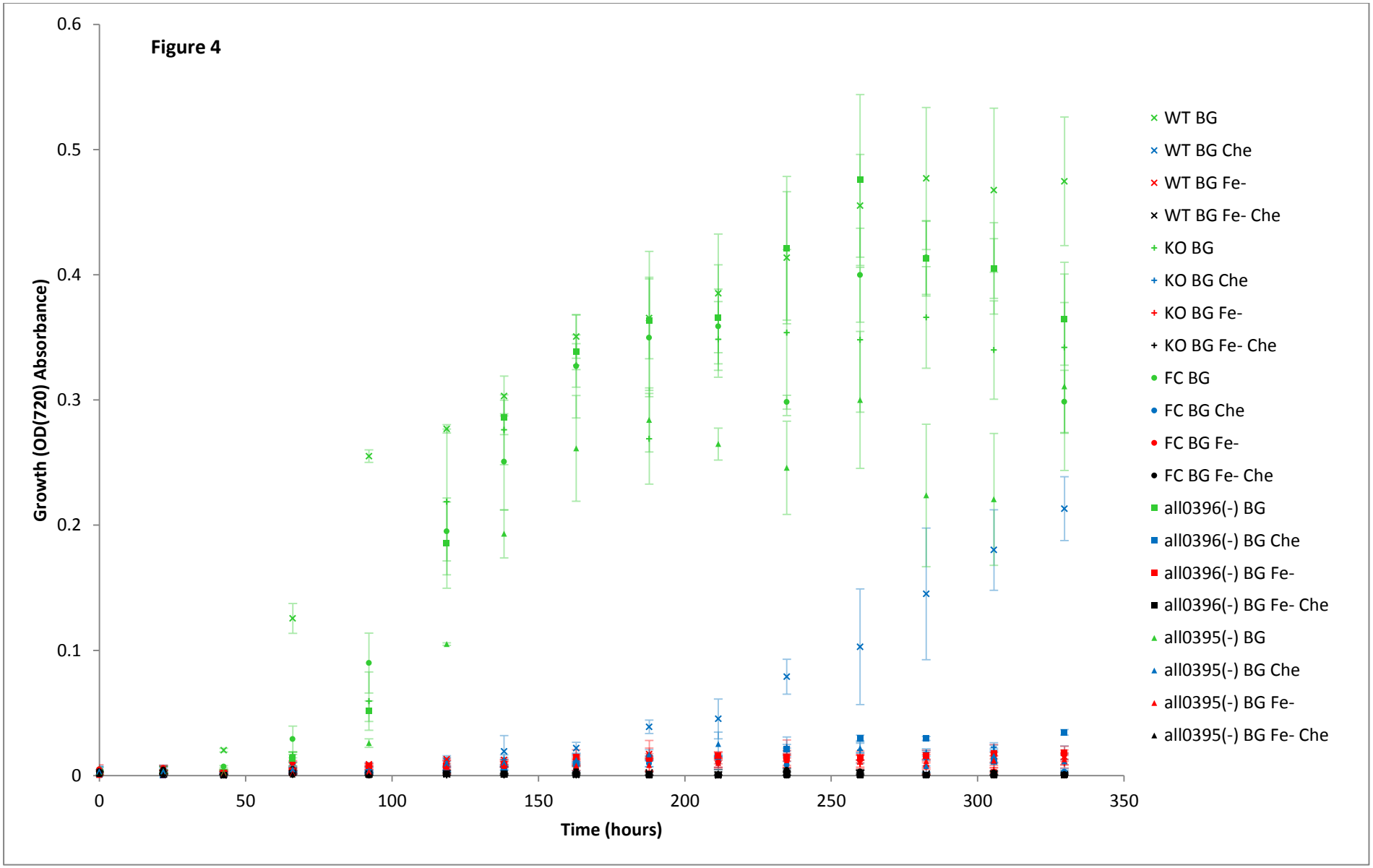

Growth (OD(720) Absorbance)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
50
100
150
200
250
300
350
Time (hours)
WT BG
WT BG Che
WT BG Fe-
WT BG Fe- Che
KO BG
KO BG Che
KO BG Fe-
KO BG Fe- Che
FC BG
FC BG Che
FC BG Fe-
FC BG Fe- Che
all0396(-) BG
all0396(-) BG Che
all0396(-) BG Fe-
all0396(-) BG Fe- Che
all0395(-) BG
all0395(-) BG Che
all0395(-) BG Fe-
all0395(-) BG Fe- Che

Figure 5

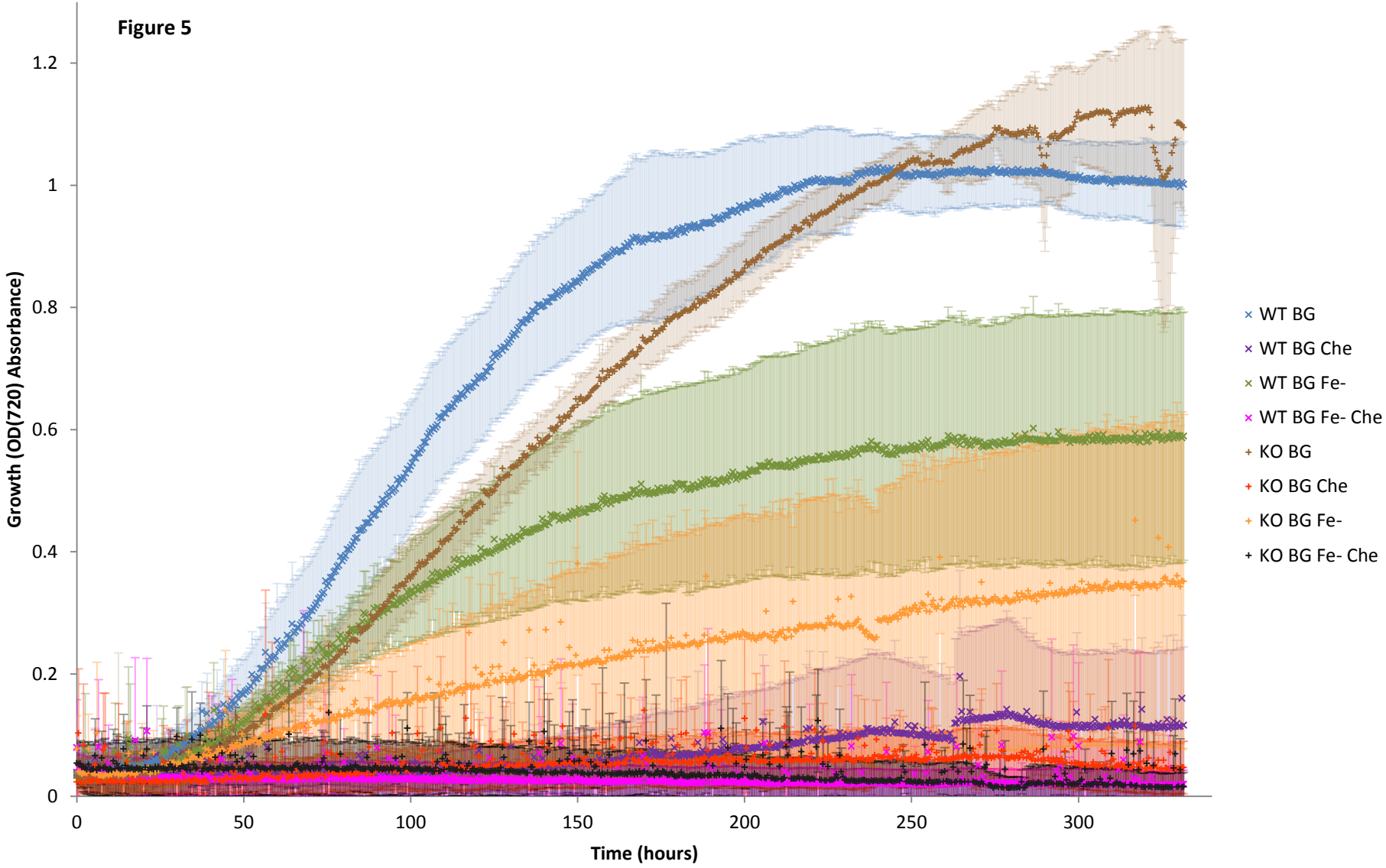

Growth (OD(720) Absorbance)
1.2
1
0.8
0.6
0.4
0.2
0
0
50
100
150
200
250
300
Time (hours)
WT BG
WT BG Che
WT BG Fe-
WT BG Fe- Che
KO BG
KO BG Che
KO BG Fe-
KO BG Fe- Che

Figure 4: Mean Growth Data for the *Anabaena* Schizokinen Mutant Strains Growth Assays Conducted Using 6-Well Plates. Treatment groups, as detailed by the key, involve five different strains including the wild type (see table 1) and four different media types (see table 2). Each point represents a mean value from three replicates; bars show 95% confidence intervals.

Figure 5: Mean Growth Data for the *Anabaena* Schizokinen Mutant Strains Growth Assays Conducted Using the Multi-Cultivator 1000. Wild type and knock-out strains were involved (see table 1) as well as four different media types (see table 2) – as detailed by the key. Each point represents a mean value from at least four replicates (see table 3); bars show 95% confidence intervals.

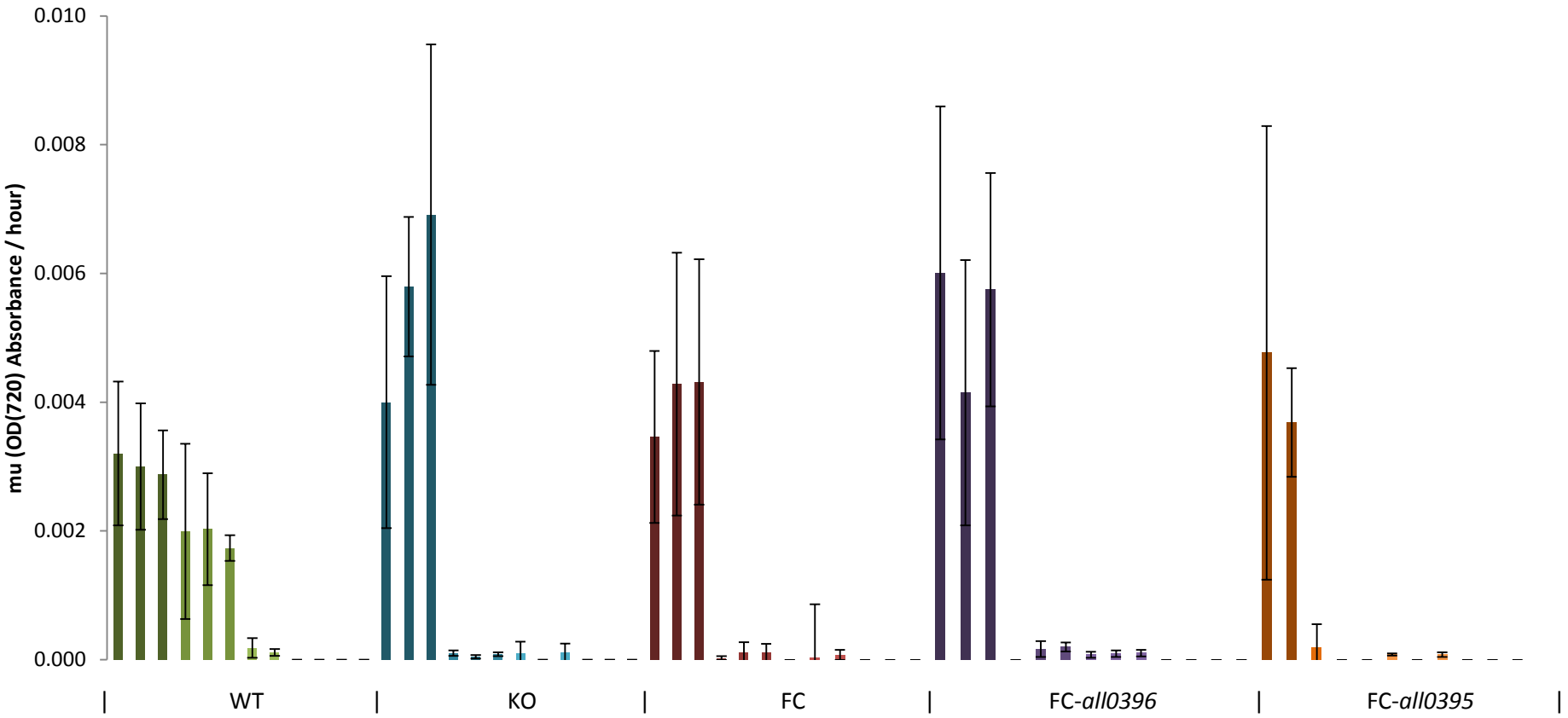


Figure 6: Bar Chart Showing Values of Mu (Maximal Growth Rate) for *Anabaena* Schizokinen Mutant Strains (6-Well Plates) Growth Assay Datasets, Fitted to Logistic Growth Curve Models. Each bar shows the value of mu for a single dataset (replicate) of each treatment; there are three replicates for each treatment. Brackets show 95% confidence intervals. Mutant strains: shades of green = wild type; shades of blue = KO; shades of maroon = FC; shades of purple = FC –*all0396*; shades of orange = FC –*all0395* (see table 1). Media types: darker to lighter shades correspond to media types 1-4 (see table 2). The absence of bars indicates in each case a failure of the given dataset to fit the logistic model.

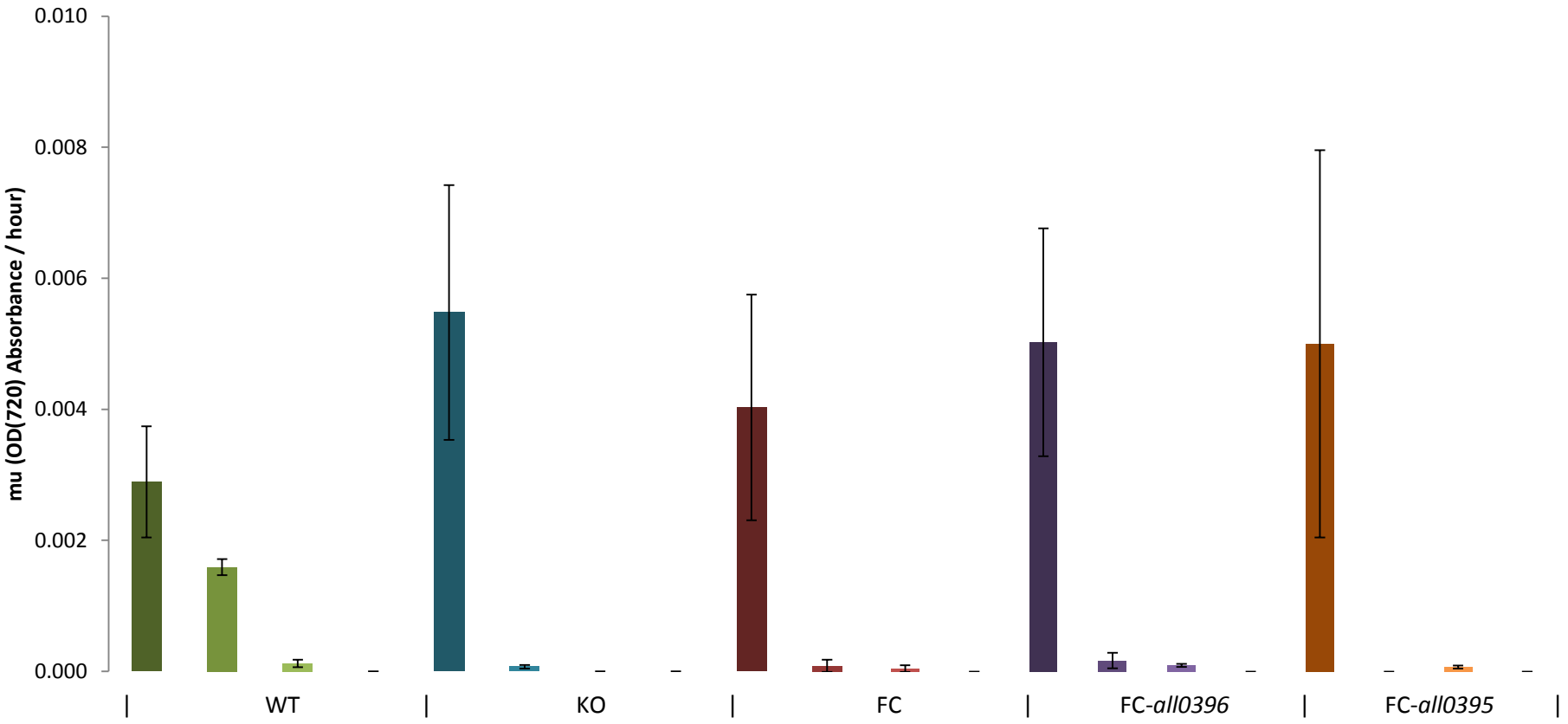


Figure 7: Bar Chart Showing Values of Mu (Maximal Growth Rate) for *Anabaena* Schizokinen Mutant Strains (6-Well Plates) Growth Assay Means, Fitted to Logistic Growth Curve Models. Each bar shows the value of mu for means of three replicates of one treatment group. Brackets show 95% confidence intervals. Mutant strains: shades of green = wild type; shades of blue = KO; shades of maroon = FC; shades of purple = FC –*all0396*; shades of orange = FC –*all0395* (see table 1). Media types: darker to lighter shades correspond to media types 1-4 (see table 2). The absence of bars indicates in each case a failure of the given dataset to fit the logistic model.

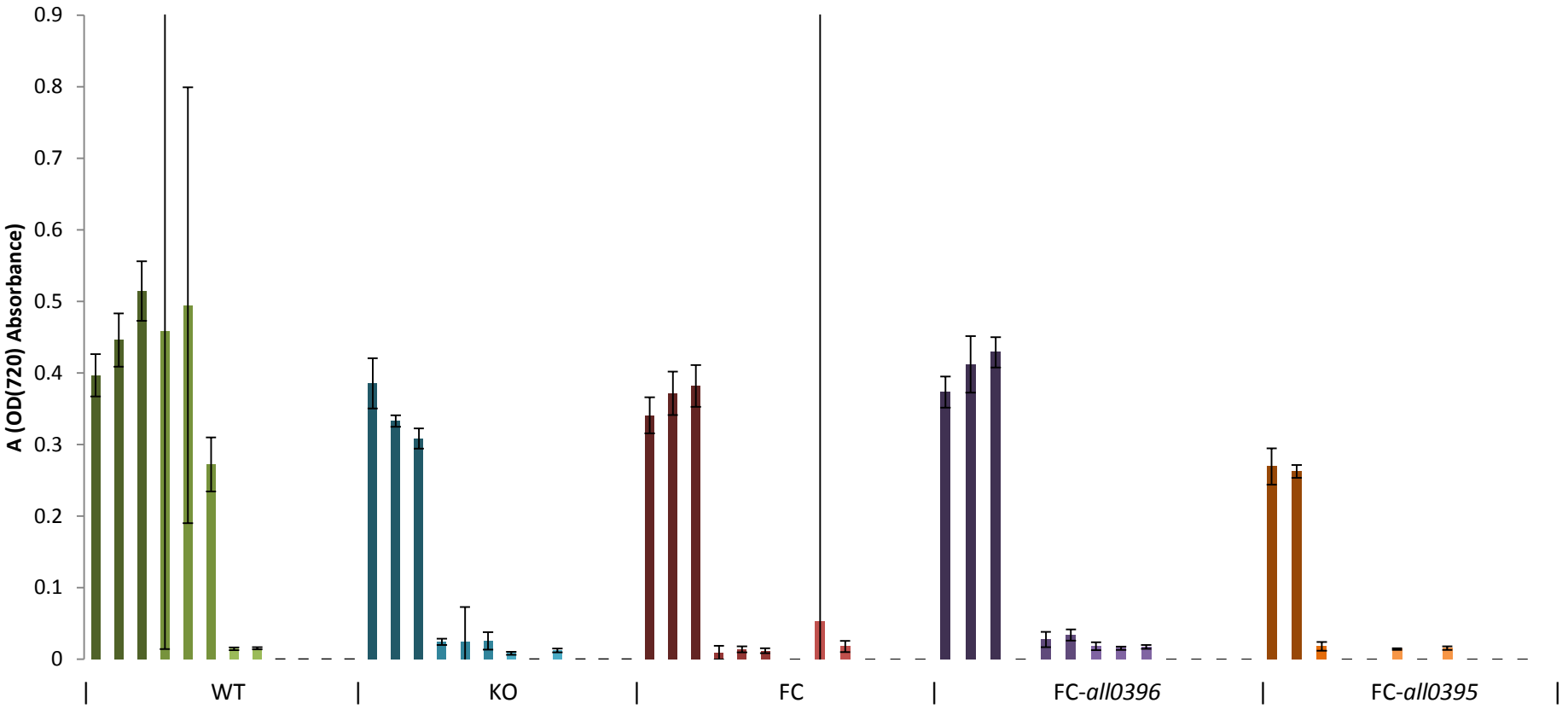


Figure 8: Bar Chart Showing Values of A (Maximum OD_{720} Absorbance Value Reached, at Plateau Phase) for *Anabaena* Schizokinen Mutant Strains (6-Well Plates) Growth Assay Datasets, Fitted to Logistic Growth Curve Models. Each bar shows the value of A for a single dataset (replicate) of each treatment; there are three replicates for each treatment. Brackets show 95% confidence intervals. Mutant strains: shades of green = wild type; shades of blue = KO; shades of maroon = FC; shades of purple = FC –*all0396*; shades of orange = FC –*all0395* (see table 1). Media types: darker to lighter shades correspond to media types 1-4 (see table 2). The absence of bars indicates in each case a failure of the given dataset to fit the logistic model.

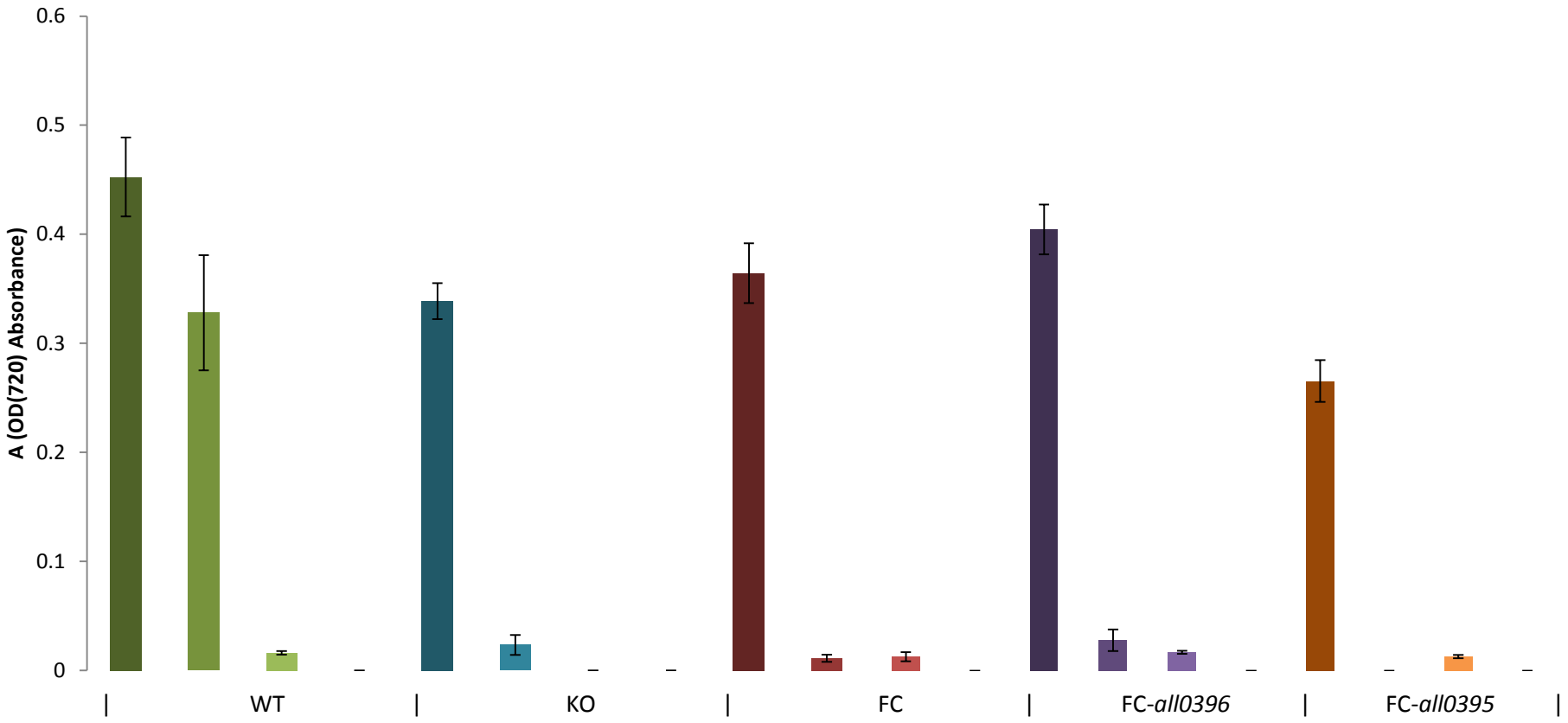


Figure 9: Bar Chart Showing Values of A (Maximum OD_{720} Absorbance Value Reached, at Plateau Phase) for *Anabaena* Schizokinen Mutant Strains (6-Well Plates) Growth Assay Means, Fitted to Logistic Growth Curve Models. Each bar shows the value of A for means of three replicates of one treatment group. Brackets show 95% confidence intervals. Mutant strains: shades of green = wild type; shades of blue = KO; shades of maroon = FC; shades of purple = FC –*all0396*; shades of orange = FC –*all0395* (see table 1). Media types: darker to lighter shades correspond to media types 1-4 (see table 2). The absence of bars indicates in each case a failure of the given dataset to fit the logistic model.

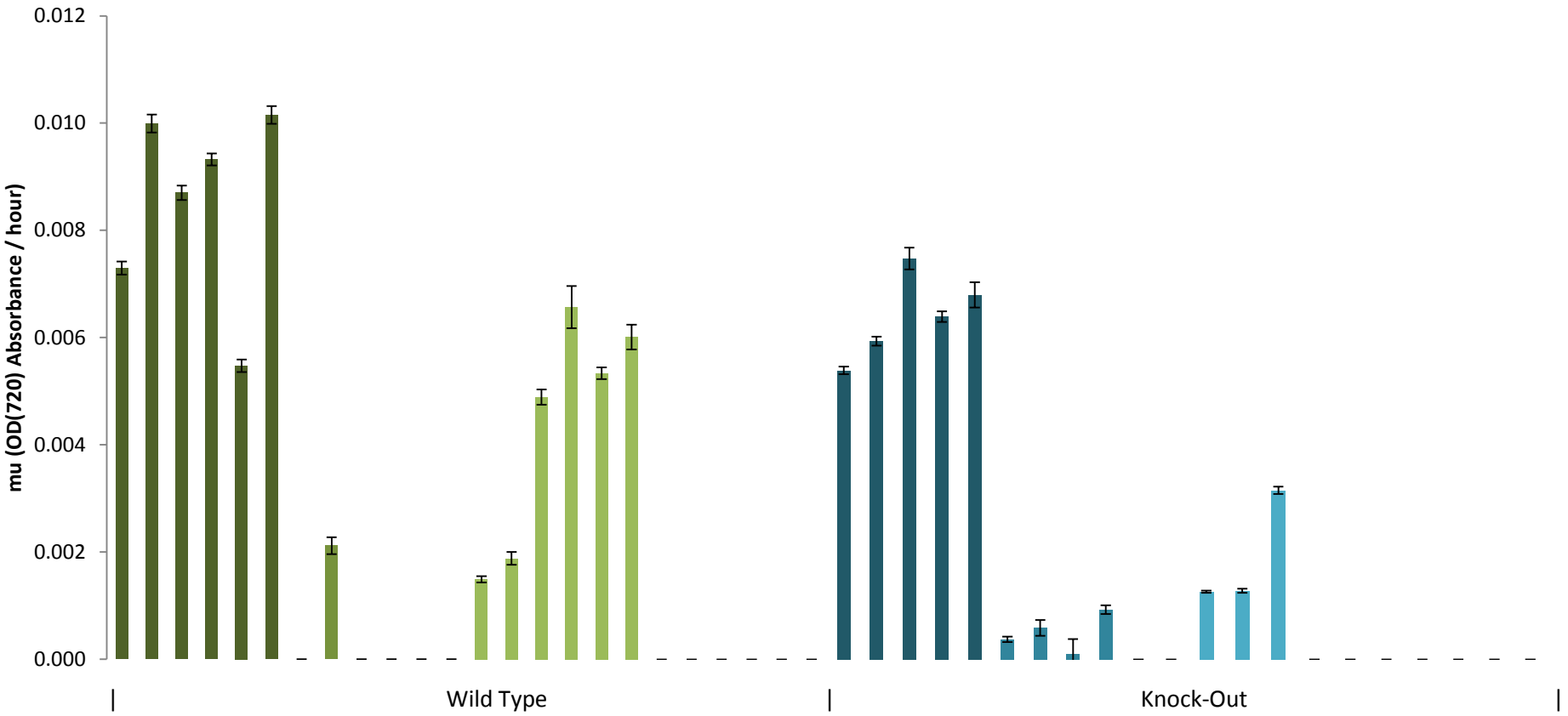


Figure 10: Bar Chart Showing Values of Mu (Maximal Growth Rate) for *Anabaena* Schizokinen Mutant Strains (MC1000) Growth Assay Datasets, Fitted to Logistic Growth Curve Models. Each bar shows the value of mu for a single dataset (replicate) of each treatment; there are at least four replicates for each treatment (detailed in table 3). Brackets show 95% confidence intervals. Mutant strains: shades of green = wild type; shades of blue = KO (see table 1). Media types: darker to lighter shades correspond to media types 1-4 (see table 2). The absence of bars indicates in each case a failure of the given dataset to fit the logistic model.

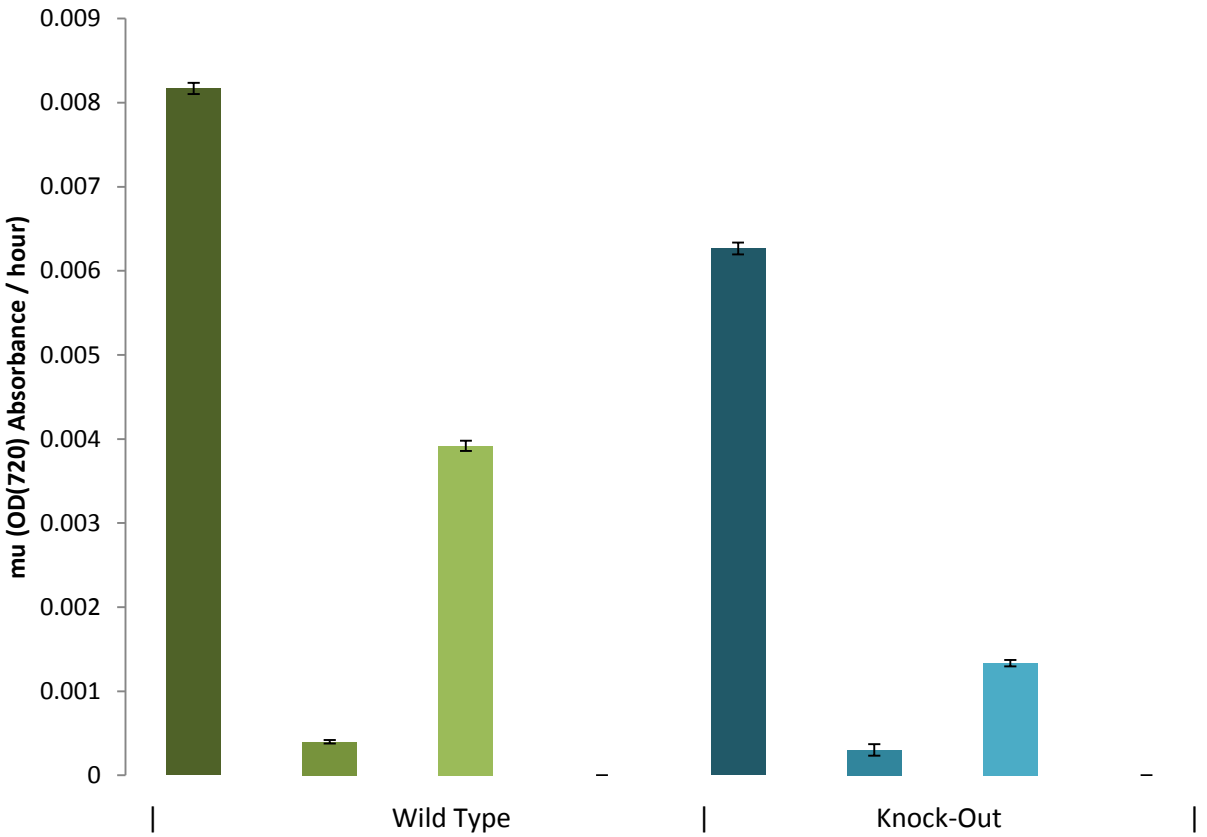


Figure 11: Bar Chart Showing Values of Mu (Maximal Growth Rate) for *Anabaena* Schizokinen Mutant Strains (MC1000) Growth Assay Means, Fitted to Logistic Growth Curve Models. Each bar shows the value of mu for means of replicates of one treatment group; there are at least four replicates for each treatment (detailed in table 3). Brackets show 95% confidence intervals. Mutant strains: shades of green = wild type; shades of blue = KO. Media types: darker to lighter shades correspond to media types 1-4 (see table 2). The absence of bars indicates in each case a failure of the given dataset to fit the logistic model.

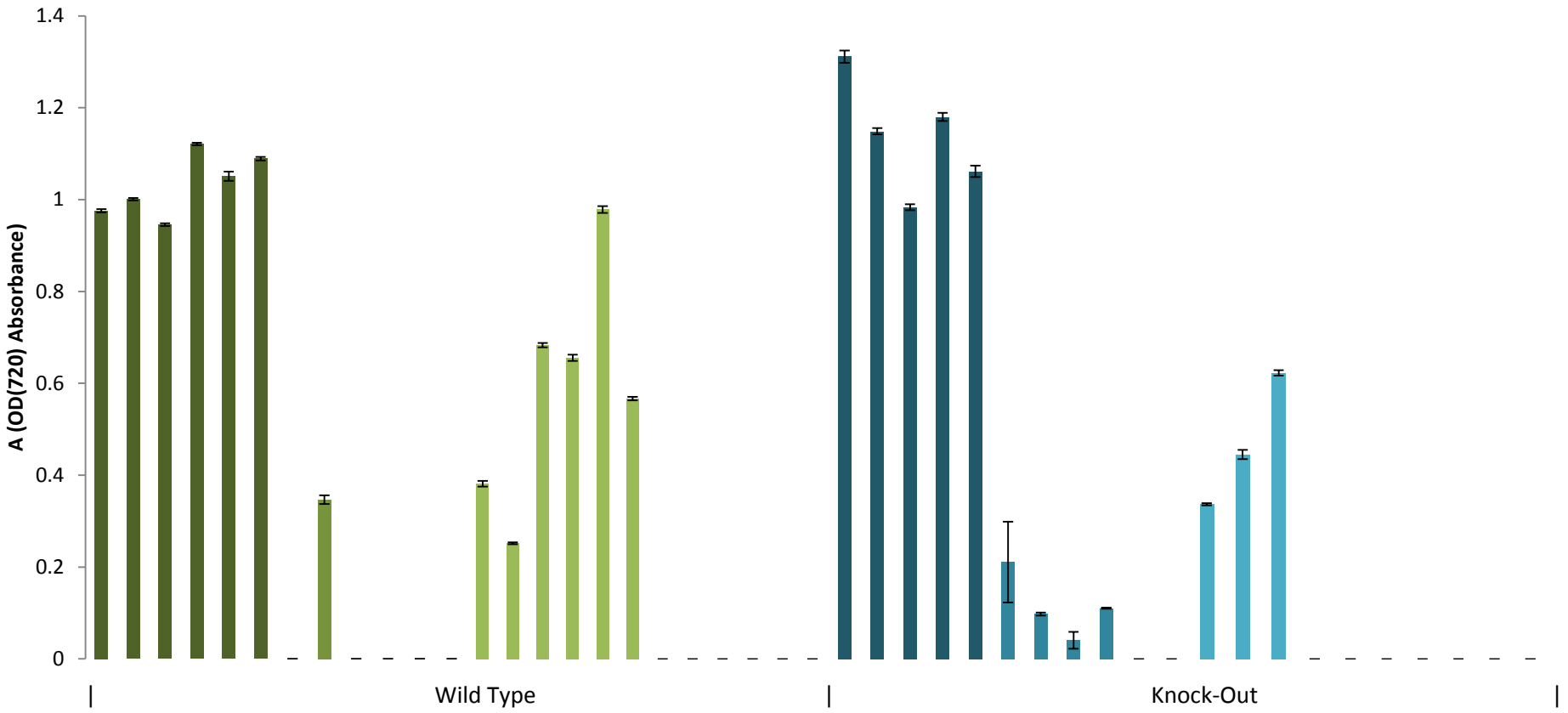


Figure 12: Bar Chart Showing Values of A (Maximum OD_{720} Absorbance Value Reached, at Plateau Phase) for *Anabaena* Schizokinen Mutant Strains (MC1000) Growth Assay Datasets, Fitted to Logistic Growth Curve Models. Each bar shows the value of A for a single dataset (replicate) of each treatment; there are at least four replicates for each treatment (detailed in table 3). Brackets show 95% confidence intervals. Mutant strains: shades of green = wild type; shades of blue = KO (see table 1). Media types: darker to lighter shades correspond to media types 1-4 (see table 2). The absence of bars indicates in each case a failure of the given dataset to fit the logistic model.

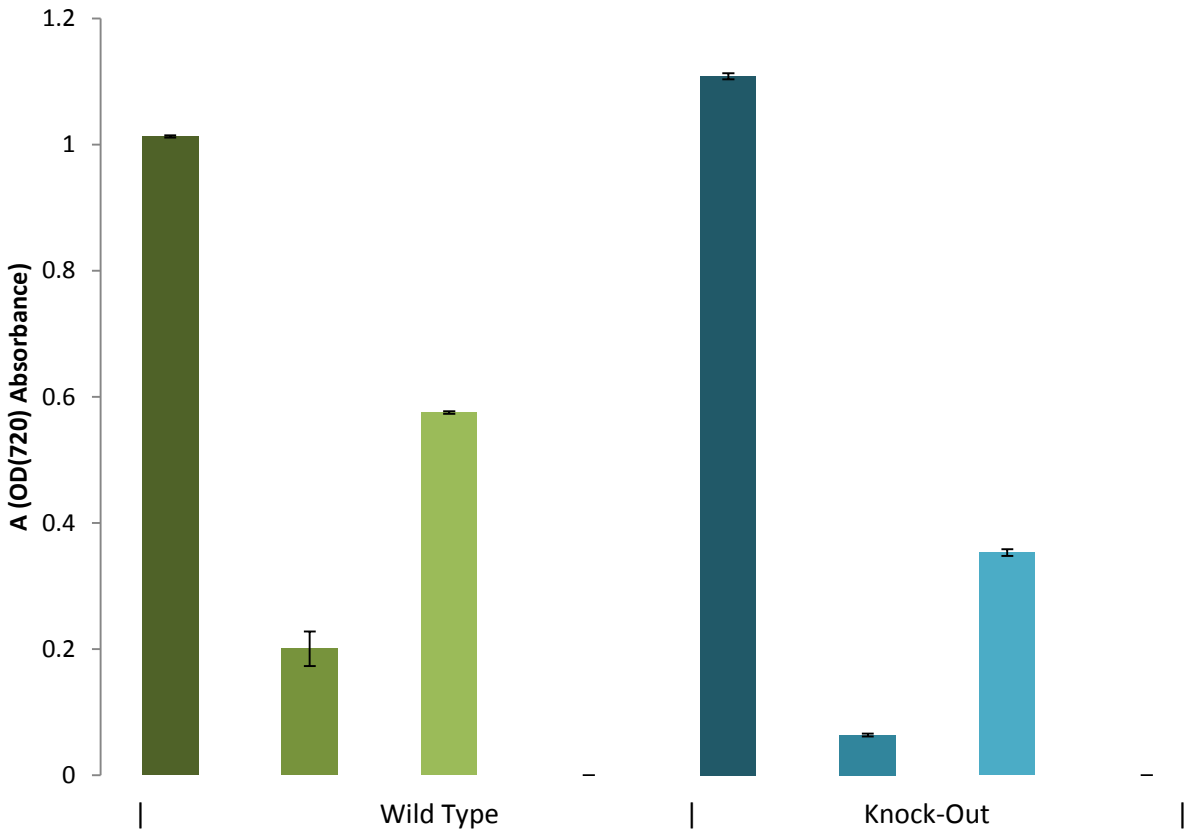


Figure 13: Bar Chart Showing Values of A (Maximum OD_{720} Absorbance Value Reached, at Plateau Phase) for *Anabaena* Schizokinen Mutant Strains (MC1000) Growth Assay Means, Fitted to Logistic Growth Curve Models. Each bar shows the value of A for means of replicates of one treatment group; there are at least four replicates for each treatment (detailed in table 3). Brackets show 95% confidence intervals. Mutant strains: shades of green = wild type; shades of blue = KO. Media types: darker to lighter shades correspond to media types 1-4 (see table 2). The absence of bars indicates in each case a failure of the given dataset to fit the logistic model.

Mean data from the ***Anabaena* schizokinen mutant strains growth assays**, conducted in 6-well plates and the MC1000 is shown in figures 4 and 5. The brackets on these figures showing 95 per cent confidence intervals in some cases are large and suggest great variation in the data. However it is worth noting that the most suitable analysis of growth assay data comes from fitting individual datasets to growth curve models [7] and from those calculating parameters of interest. This was performed for growth assay data here. Full growth curve analyses using the Grofit package are detailed in appendix D – with assessments of model fits (appendix tables D(i-iv)), full R outputs and fitted growth curves for individual datasets as well as means (appendix figures D(i-ii) and D(v-vi)). Calculated from fitted growth curves, parameters of lambda – duration of lag phase, mu – maximal growth rate during logarithmic phase, and A – maximum absorbance value reached at plateau phase [7] – are shown in appendix figures D(iii-iv) and D(vii-viii), figures 6, 7, 10, and 11, and figures 8, 9, 12, and 13 respectively for individual and mean data from the 6-well plate and MC1000 – conducted assays. In both cases, values for lambda are highly variable with large confidence intervals – this being the main reason for the great variation shown in mean growth assay data graphs in figures 4 and 5. The logistic model was selected in all cases as the most suitable model for analysis based on fit.

From growth assay analyses it is clear that different media types (see table 2) produced notable differences in growth performance for all strains. Media type 4 – BG-11 without ferric ammonium citrate and containing 50µM of the chelator 2,2'-dipyridyl – was hostile to the extent that in every single case no growth was observed, resulting in failures of all datasets for growth assays using this media to fit any Grofit curve models (see appendix D). Also unsurprisingly, media type 1 – BG-11 with ferric ammonium citrate and lacking the chelator – was the most encouraging of cyanobacterial growth. The greatest maximal growth rates and plateau absorbance values were achieved by all strains in this media (see figures 6-13). In almost all cases, in media types 2 and 3 (BG-11 media containing both ferric ammonium citrate and 2,2-dipyridyl, and BG-11 media without both), strains performed better than in media type 4 and worse than in media type 1 (see figures 6-13), but varied in their growing abilities regarding comparisons between types 2 and 3 (see figures 6-9). More data is required to gain higher confidence in the difference for the full complement strain and strains FC-*all0396* and FC-*all0395* under these two media types – 6-well plate assay analyses mostly show no significant differences in values of mu or A for the full complement or FC-*all0396* strains (FC mu: 0.0000266 ± 0.0000295, 0.000121 ± 0.000152, 0.000109 ± 0.000138 vs. 0.0000381 ± 0.000827, 0.0000780 ± 0.0000797; FC means mu: 0.0000857 ± 0.0000942 vs. 0.0000472 ± 0.0000498; FC A: 0.00915 ± 0.00960, 0.0138 ± 0.00411, 0.0119 ± 0.00352 vs. 0.0533 ± 1.48, 0.0182 ± 0.00775; FC means A: 0.0112 ± 0.00334 vs. 0.0128 ± 0.00424; FC-*all0396* mu: 0.000196 ± 0.000359 vs. 0.0000833 ± 0.0000168, 0.0000813 ± 0.0000356; FC-*all0396* A: 0.0185 ± 0.00615 vs. 0.0145 ± 0.00120, 0.0158 ± 0.00241 for media types 2 and 3 respectively – figures 6-9), whilst values for mu and A are greater for media type 3 than type 2 for FC-*all0395* (FC-*all0395* mu: 0.0001687611 ± 0.000124, 0.000200 ± 0.0000700 vs. 0.0000802 ± 0.0000451, 0.0000986 ± 0.0000509, 0.000106 ± 0.0000520; FC-*all0395* means mu: 0.000169 ± 0.000118 vs. 0.0000952 ± 0.0000248; FC-*all0395* A: 0.0279 ± 0.0106, 0.0342 ± 0.00783 vs. 0.0186 ± 0.00564, 0.0156 ± 0.00224, 0.0177 ± 0.00261; FC-*all0395* means A: 0.0279 ± 0.00983 vs. 0.0169 ± 0.00143 for media types 2 and 3 respectively – figures 6-9). MC1000-conducted growth assays allowed this to be assessed with more confidence for the wild type and knock-out strains – and unlike the 6-well plate analyses, MC1000 analyses mostly show significantly greater values for mu and A in media type 3 than type 2 for both strains (WT mu: 0.0102 ± 0.000166, 0.00212 ± 0.000158 vs. 0.00149 ± 0.0000578, 0.00188 ± 0.000121, 0.00489 ± 0.000140, 0.00657 ± 0.000395, 0.00533 ± 0.000108; WT means mu: 0.000398 ± 0.0000209 vs. 0.00392 ± 0.0000604; WT A: 1.09 ± 0.00360, 0.347 ± 0.00941 vs. 0.381 ± 0.00594, 0.252 ± 0.00223, 0.683 ± 0.00470, 0.656 ± 0.00678, 0.978 ± 0.00763; WT means A: 0.201 ± 0.0273 vs. 0.575 ± 0.00214; KO mu: 0.000376 ± 0.0000527, 0.000590 ± 0.000147, 0.0000995 ± 0.000283, 0.000930 ± 0.0000810 vs. 0.00126 ± 0.0000188, 0.00128 ± 0.0000367, 0.00315 ± 0.0000698; KO means mu: 0.000301 ± 0.0000675 vs. 0.00133 ± 0.0000374; KO A: 0.211 ± 0.0879, 0.0980 ± 0.00323, 0.0408 ± 0.0184, 0.111 ± 0.001 vs. 0.337 ± 0.002, 0.445 ± 0.0103, 0.623 ± 0.00591; KO means A: 0.0637 ± 0.00228 vs. 0.353 ± 0.00537; for media types 2 and 3 respectively – figures 10-13). This indicates that for the wild type and knock-out strains, the presence of 50µM chelator

2,2'-dipyridyl has a greater negative effect on overall growth performance (both maximal growth rate and growth achieved) than absence of ferric ammonium citrate in BG-11 media.

As previously stated, the wild type and knock-out strains, like the others, performed better in media type 1 than in all other media types. Comparing these two strains with each other was conducted in both the 6-well plate growth assays and with greater sampling and confidence in the MC1000 growth assays (figures 1 and 3). Results from these show slight differences – for example in the 6-well plate assays, the wild type had a greater absorbance at plateau than the knock-out (WT A vs. KO A: 0.397 ± 0.0296, 0.446 ± 0.0373, 0.514 ± 0.0415 vs. 0.386 ± 0.0350, 0.333 ± 0.00796, 0.308 ± 0.0140; WT means A vs. KO means A: 0.453 ± 0.0361 vs. 0.339 ± 0.0165 for media type 1 – see Figures I and J), but the opposite was true for the MC1000 assays (WT A vs. KO A: 0.976 ± 0.00369, 1.00 ± 0.00289, 0.946 ± 0.00289, 1.12 ± 0.00277, 1.05 ± 0.0101, 1.09 ± 0.00360 vs. 1.31 ± 0.0133, 1.15 ± 0.00680, 0.983 ± 0.00657, 1.18 ± 0.00862, 1.06 ± 0.0124; WT means A vs. KO means A: 1.01 ± 0.00182, 1.11 ± 0.00486 for media type 1 – figures 12 and 13); and whilst the knock-out mutant strain had a greater maximal growth rate in the 6-well plate assays (WT mu vs. KO mu: 0.00321 ± 0.00112, 0.00300 ± 0.000982, 0.00287 ± 0.000689 vs. 0.00400 ± 0.00196, 0.00579 ± 0.00108, 0.00691 ± 0.00264; WT means mu vs. KO means mu: 0.00289 ± 0.000849 vs. 0.00548 ± 0.00194 for media type 1 – see Figures G and H), again results from the MC1000 contradicted this (WT mu vs. KO mu: 0.00730 ± 0.000122, 0.00999 ± 0.000169, 0.00870 ± 0.000134, 0.00932 ± 0.000113, 0.00547 ± 0.000116, 0.0102 ± 0.000166 vs. 0.00539 ± 0.0000715, 0.00593 ± 0.0000832, 0.00747 ± 0.000201, 0.00639 ± 0.0000980, 0.00680 ± 0.000237; WT means mu vs. KO means mu: 0.00817 ± 0.0000673 vs. 0.00626 ± 0.0000706 for media type 1 – figures 10 and 11). In media types 2 and 3, in all growth assays and regarding both parameters, the wild type strain performed better than the knock-out mutant (see figures 6-13).

A surprising result from the 6-well plate growth assay analyses was that the full complement mutant strain did not appear to fully live up to its name. In media type 2, the introduction of the RSF1010 plasmid containing the schizokinen operon into the knock-out mutant (i.e. the full complement mutant) did not restore maximal growth rates or maximum absorbance values at plateau to wild type levels, with significantly-lower values for mu (WT mu vs. FC mu: 0.00199 ± 0.00136, 0.00203 ± 0.000869, 0.00173 ± 0.000199 vs. 0.0000266 ± 0.0000295, 0.000121 ± 0.000152, 0.000109 ± 0.000138; WT means mu vs. FC means mu: 0.00159 ± 0.000123 vs. 0.0000857 ± 0.0000942 for media type 2 – see Figures G and H) and A (WT A vs. FC A: 0.458 ± 0.444, 0.495 ± 0.304, 0.272, 0.0379 vs. 0.00915 ± 0.00960, 0.0138 ± 0.00411, 0.0119 ± 0.00352; WT means A vs. FC means A: 0.328 ± 0.0528 vs. 0.0112 ± 0.00333 for media type 2 – figures 8 and 9) in the full complement strain than the wild type, more similar to those of the knock-out mutant (KO mu: 0.000102 ± 0.0000425, 0.0000440 ± 0.0000272, 0.0000837 ± 0.0000297; KO means mu: 0.0000708 ± 0.0000274; KO A: 0.0243 ± 0.00434, 0.0250 ± 0.0481, 0.0256 ± 0.0123; KO means A: 0.0234 ± 0.00912 for media type 2 – figures 6-9). However, values for these parameters were restored in the case of media type 3 (WT mu vs. FC mu: 0.000184 ± 0.000153, 0.000113 ± 0.0000535 vs. 0.0000381 ± 0.000827, 0.0000780 ± 0.0000797; WT means mu vs. FC means mu: 0.000123 ± 0.0000581 vs. 0.0000472 ± 0.0000498; WT A vs. FC A: 0.0147 ± 0.00185, 0.0153 ± 0.00146 vs. 0.0534 ± 1.48, 0.0182 ± 0.00775; WT means A vs. FC means A: 0.0161 ± 0.00162 vs. 0.0128 ± 0.00424 for media type 3 – figures 6-9).

The Chrome Azurol S assay (figure 14) showed colour changes and detection of siderophore secretion by all five strains including the knock-out. Considering that more than one siderophore is known for *Anabaena* 7120 (e.g. Jeanjean *et al.* 2008), this is not surprising. Unfortunately however, any approximate quantification of siderophore secretion using this method was not possible.

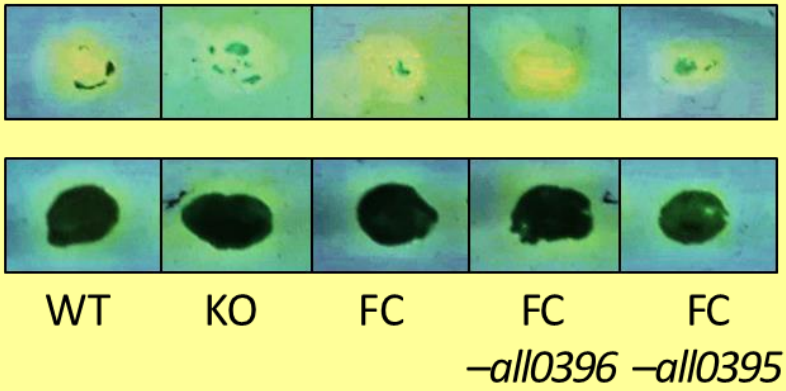


Figure 14: Chrome Azurol S Assays. Four schizokinen operon mutant strains are shown along with the wild type, as labelled. Bottom row: prior to colony removal; top row: after cells have been removed, showing blue-yellow colour changes in the media.

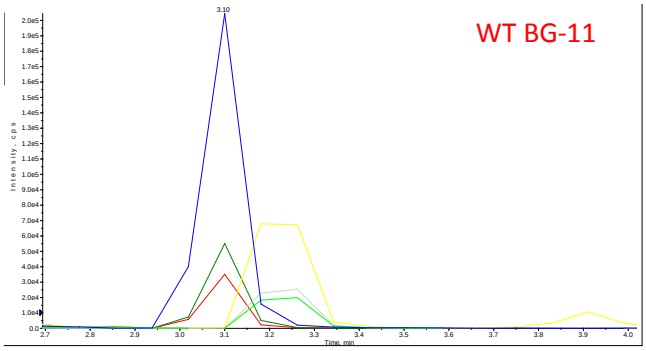
Liquid chromatography–mass spectrometry of trypsin-digested protein extractions from wild type, knock-out, full complement, FC-*all3096* and FC-*all0395* strains provided some insights into the expression of proteins of interest. The original list of transitions (see appendix E) from the tryptic peptides of 10 proteins of interest [8] was refined after initial MRM runs to a new schedule of retention times focussing on the most promising proteins for detection – those encoded by genes *All0390*, *All0394*, *All0396*, *All4001*, *All2650* and the RuBisCO large subunit [8]. Table G lists the detected peptides shown by figures in this results section.

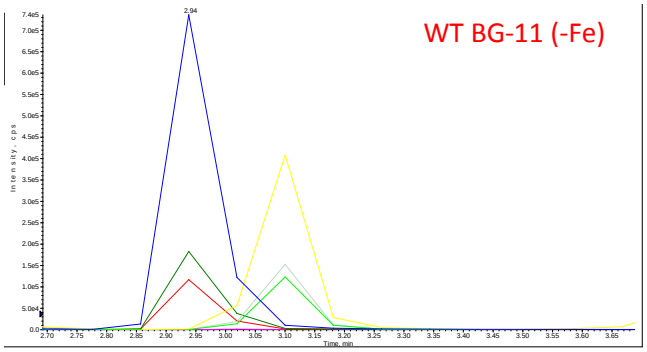
The positive control, RuBisCO large subunit protein, is shown to be expressed by all ten samples. What is also shown by the aligned MRM graphs in appendix figure E(i) as well as the aligned MRM graphs for the peptide EGNDVIR in figure 15 is that qualities of trypsin digests varied between samples. This means that data obtained from these analyses can support the expression of given protein-encoding genes, but is not sufficient to provide quantitative comparisons between samples. Given the lack of detection of the photosystem II chlorophyll A-binding protein (*All4001*) in all samples (appendix figure E(ii)) – a control for iron deficient conditions – qualitative nor quantitative assessments on expression in different media types could be made. Predicted transitions for the ATP-binding cassette transporter of another siderophore in *Anabaena* 7120 [6; 8] encoded by *All2650* were included in the original transition list and later schedule, although peptides were not detected.

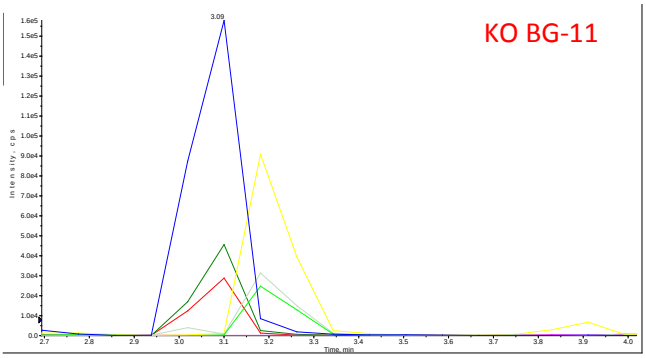
Table 6: Details of Detected Tryptic Peptides of Proteins of Interest [8; 15], considered in figures 15-25.

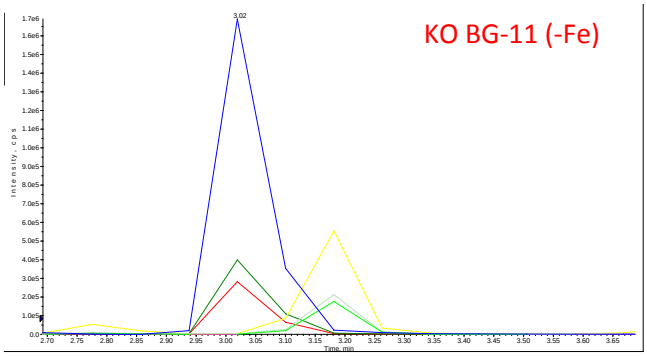
Protein	Locus	Tryptic Peptide Sequence	Retention Time (Schedule) (mins)	Transitions (3s.f.)	
				Predicted Parent Ion m/z (Da)	Predicted Daughter Ions m/z (Da)
RuBisCO Large Subunit	*Alr1524*	EGNDVIR	3.4	402	673; 616; 502
N4-acetyl-N4-hydroxy-1-aminopropane ligase	*All0394*	QEILQDLGLVGR	23.4	671	971; 857; 729
		VDNQFK	2.6	376	651; 536; 422
Diaminobutyrate–2-oxoglutarate transaminase	*All0396*	QVLSVLSQVS	30.4	530	832; 719; 632
		AIGGSLPLSVVLYNK	12.8	766	1230; 1150; 1030; 936; 822
Schizokinen Siderophore Synthase	*All0390*	DFVDDVNISR	16.8	590	917; 818; 703
		ETILSYQSR	10.8	549	753; 640; 553
		FELFNLLAPQFTK	32.4	784	1030; 804; 620

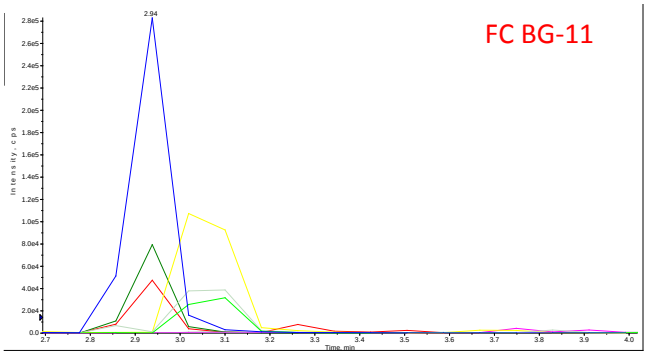
Interestingly, figures 16 and 17, 18 and 19, and 20, 21 and 22 show the detection of tryptic peptides from N4-acetyl-N4-hydroxy-1-aminopropane ligase (*All0394*), diaminobutyrate–2-oxoglutarate transaminase (*All0396*), and schizokinen siderophore synthase (*All0390*) respectively – in the full complement and FC-*all0396* (with the exception of *All0396*, as expected) strain samples. (Full MRM scans highlighting these proteins of interest are shown in appendix figures E(iii-v).) The identities of detected tryptic peptides – with sequences DFVDDVNISR, ETILSYQSR, and FELFNLLAPQFTK – from schizokinen siderophore synthase (*All0390*) were confirmed by an enhanced product ion scan (see figures 23, 24, and 25 respectively). Here, the spectra showed the presence of 6 out of 9, 6 out of 8, and 6 out of 12 predicted Y ions with m/z ratios over 200 (according to the ProSight PTM® Ion Predictor https://prosightptm.northwestern.edu/ionpredictor/) for the three peptides respectively (see figures and table 7). Therefore there is evidence of a reasonable level of expression of these schizokinen synthesis operon genes (particularly *All0390*) by the full complement, suggesting that the failures to restore growth performances to the level of the wild type by introducing the RSF1010 plasmid containing the operon to the knock-out mutant, as described earlier, was not caused by poor levels of expression, at least for the genes considered here.

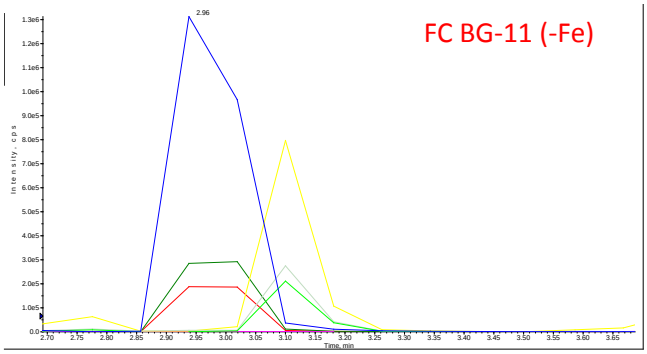

WT BG-11

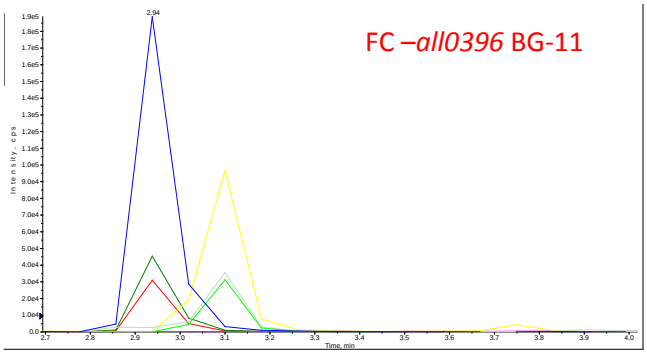

WT BG-11 (-Fe)

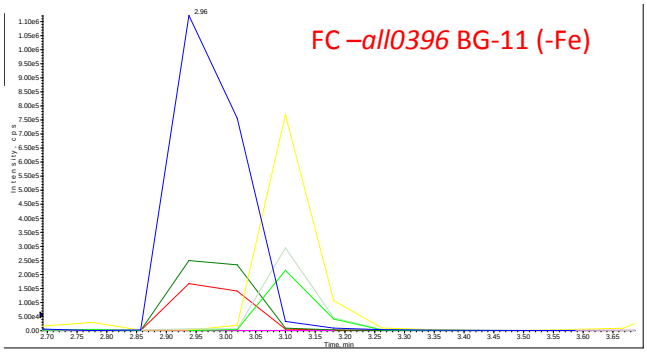

KO BG-11

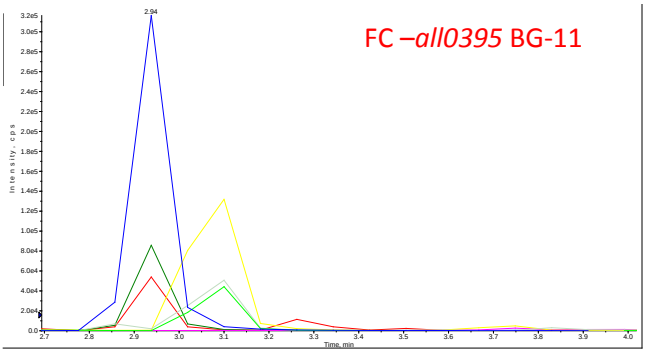

KO BG-11 (-Fe)


FC BG-11


FC BG-11 (-Fe)


FC –all0396 BG-11


FC –all0396 BG-11 (-Fe)


FC –all0395 BG-11

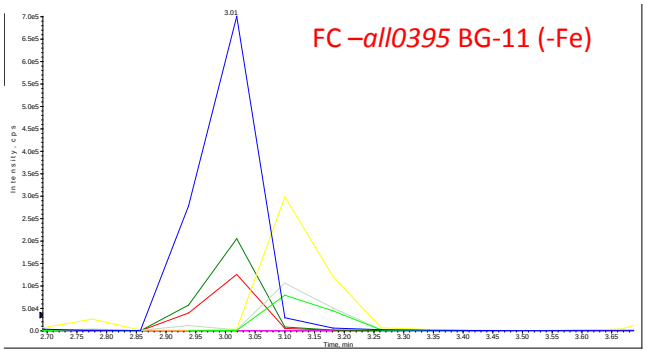

FC –all0395 BG-11 (-Fe)

Figure 15: Aligned Multiple Reaction Monitoring Mass Spectrometry Graphs Showing the Tryptic Peptide EGNDVIR of the RuBisCO Large Subunit Protein at Given Retention Times (X Axis) and Intensities (Y Axis), for the Four Schizokinen Operon Mutant Strains and the Wild Type, Exposed to BG-11 Media with and without Ferric Ammonium Citrate. The retention time predicted on the scheduled transition list for this peptide was 3.4 minutes. Intensities shown (maximum values of y axes), from WT BG-11 – FC-*all0395* BG-11 (-Fe): $2.0e^5$, $7.4e^5$, $1.6e^5$, $1.7e^6$, $2.8e^5$, $1.3e^6$, $1.9e^5$, $1.1e^6$, $3.2e^5$, $7.0e^5$.

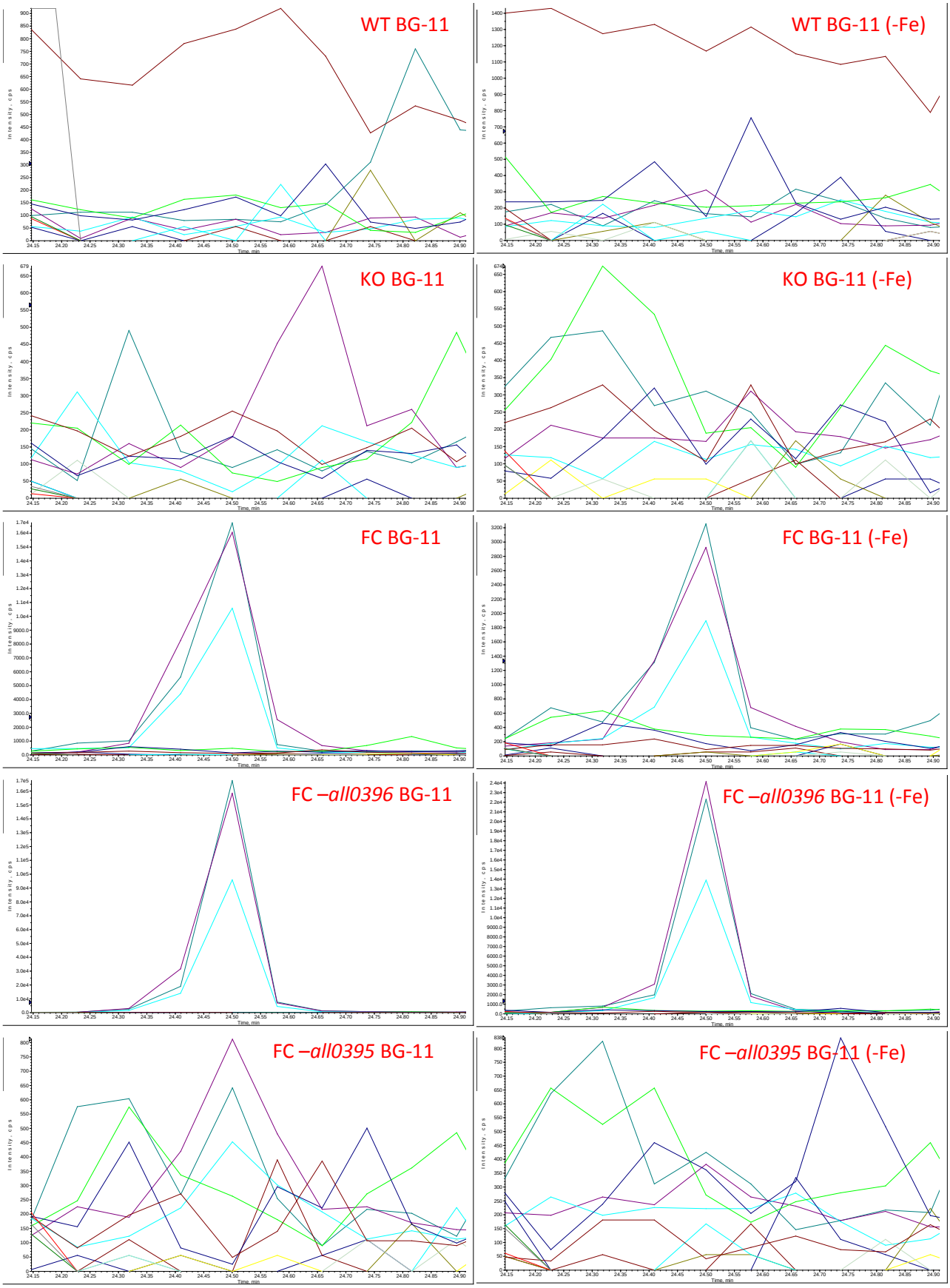

WT BG-11
WT BG-11 (-Fe)
KO BG-11
KO BG-11 (-Fe)
FC BG-11
FC BG-11 (-Fe)
FC –all0396 BG-11
FC –all0396 BG-11 (-Fe)
FC –all0395 BG-11
FC –all0395 BG-11 (-Fe)

Figure 16: Aligned Multiple Reaction Monitoring Mass Spectrometry Graphs Showing the Tryptic Peptide QEILQDLGLVGR of the N4-acetyl-N4-hydroxy-1-aminopropane Ligase Protein (*All0394*) at Given Retention Times (X Axis) and Intensities (Y Axis), for the Four Schizokinen Operon Mutant Strains and the Wild Type, Exposed to BG-11 Media with and without Ferric Ammonium Citrate. The retention time predicted on the scheduled transition list for this peptide was 23.4 minutes. Intensities shown (maximum values of y axes), from FC BG-11 – FC-*all0396* BG-11 (-Fe): $1.7e^4$, 3200, $1.7e^5$, $2.4e^4$.

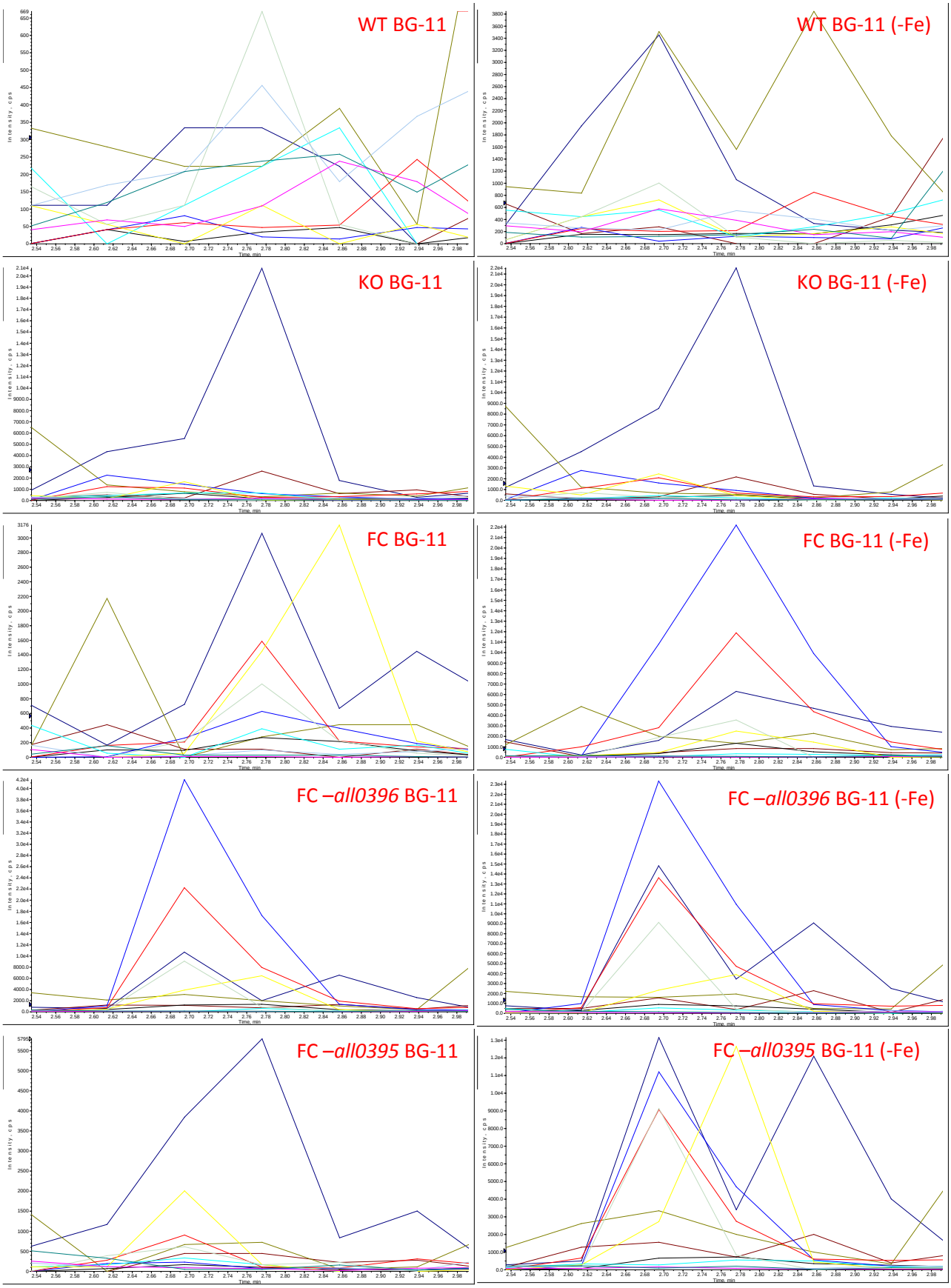
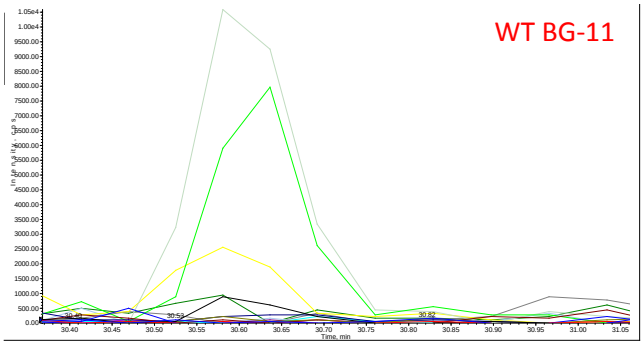
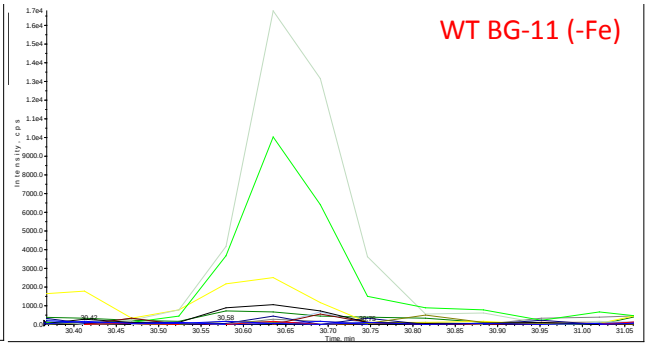
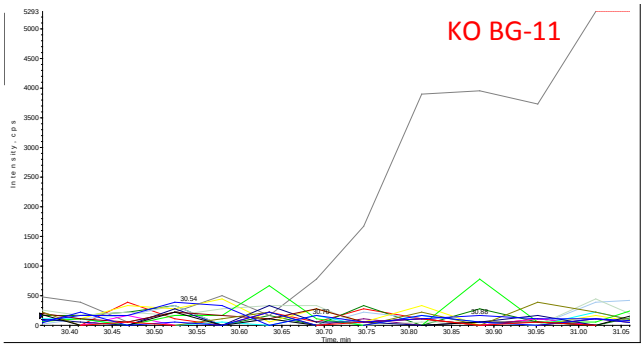
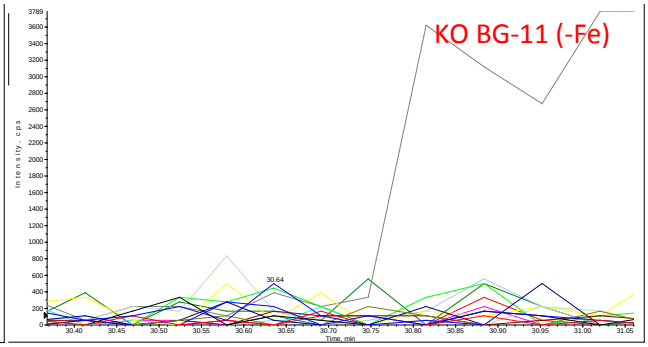

WT BG-11
WT BG-11 (-Fe)
KO BG-11
KO BG-11 (-Fe)
FC BG-11
FC BG-11 (-Fe)
FC –all0396 BG-11
FC –all0396 BG-11 (-Fe)
FC –all0395 BG-11
FC –all0395 BG-11 (-Fe)

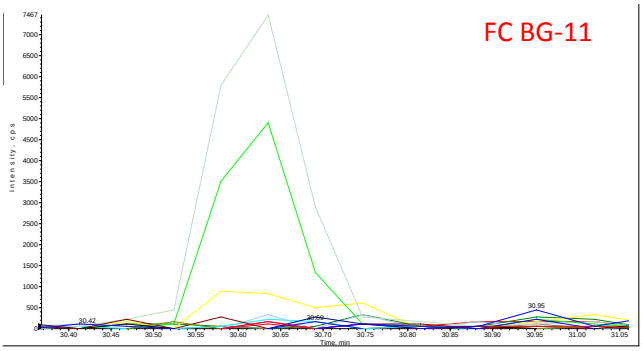
Figure 17: Aligned Multiple Reaction Monitoring Mass Spectrometry Graphs Showing the Tryptic Peptide VDNQFK of the N4-acetyl-N4-hydroxy-1-aminopropane Ligase Protein (*All0394*) at Given Retention Times (X Axis) and Intensities (Y Axis), for the Four Schizokinen Operon Mutant Strains and the Wild Type, Exposed to BG-11 Media with and without Ferric Ammonium Citrate. The retention time predicted on the scheduled transition list for this peptide was 2.6 minutes. Intensities shown (maximum values of y axes), from FC BG-11 – FC-*all0396* BG-11 (-Fe), and FC-*all0395* BG-11 (-Fe): 3176, $2.2e^4$, $4.2e^4$, $2.3e^4$, $1.3e^4$.

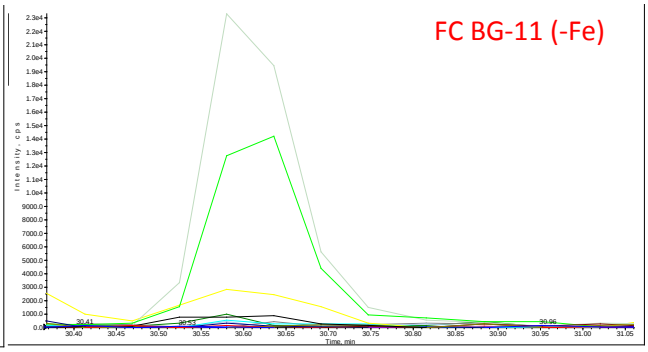

WT BG-11

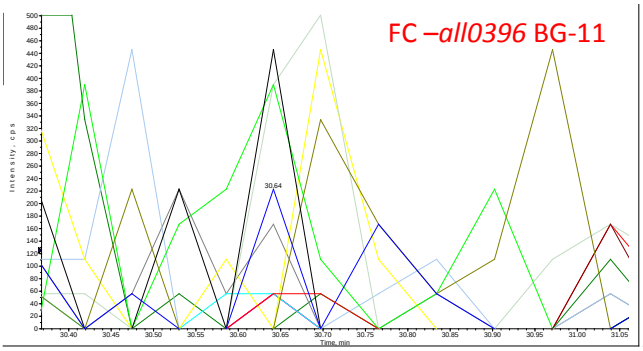

WT BG-11 (-Fe)

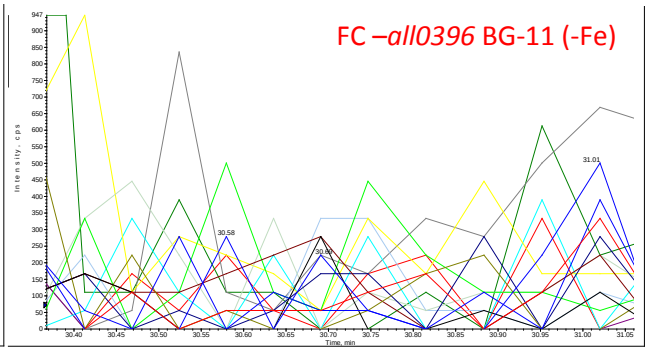

KO BG-11

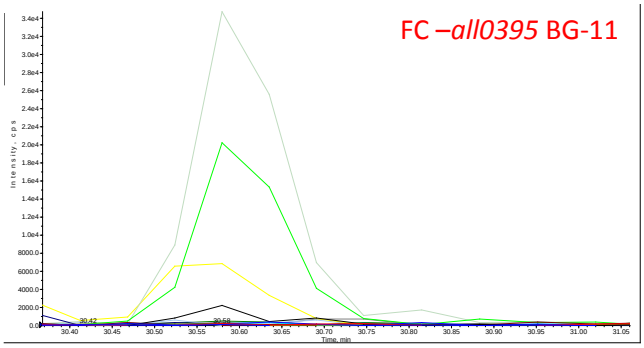

KO BG-11 (-Fe)


FC BG-11


FC BG-11 (-Fe)


FC –all0396 BG-11


FC –all0396 BG-11 (-Fe)


FC –all0395 BG-11

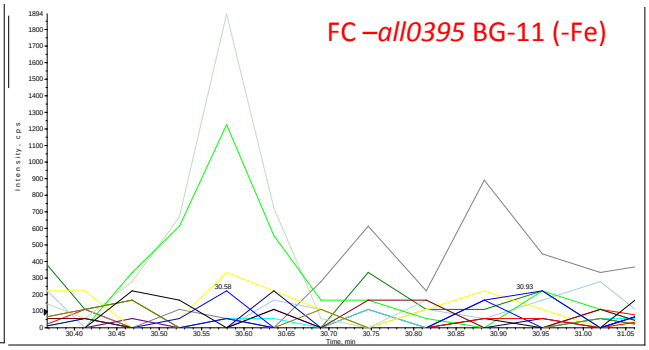
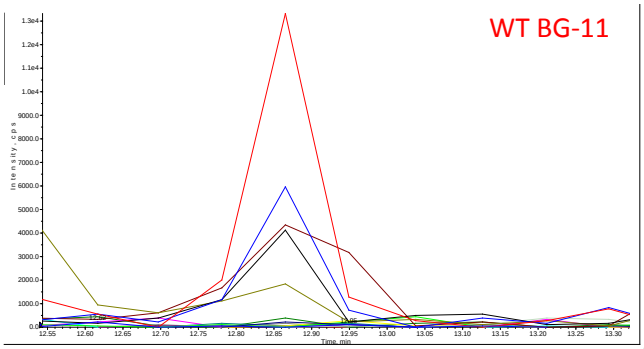
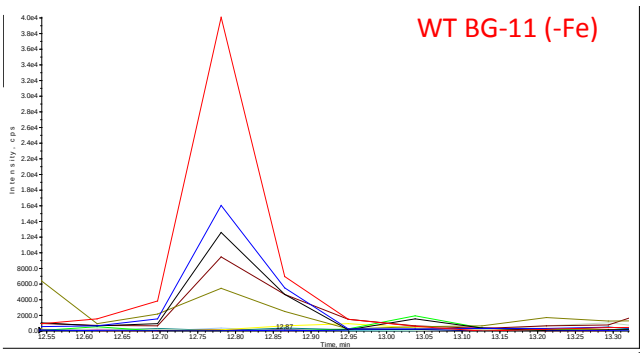
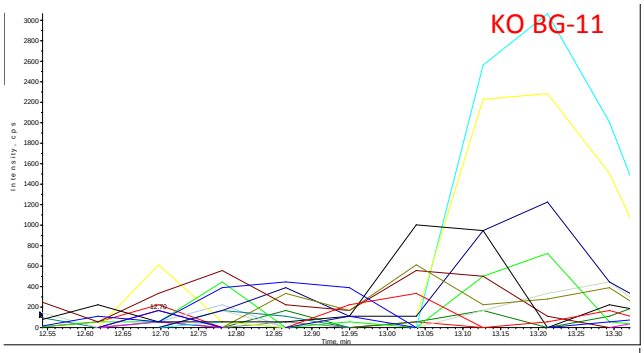
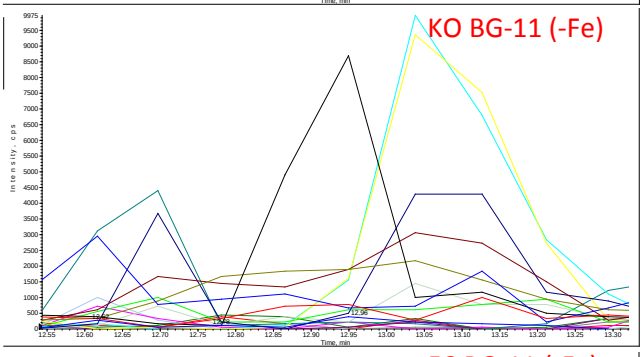

FC –all0395 BG-11 (-Fe)

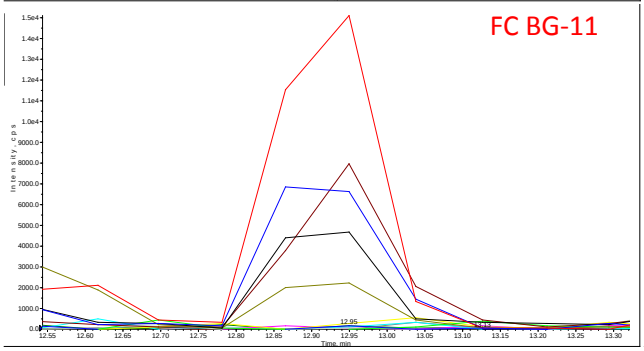
Figure 18: Aligned Multiple Reaction Monitoring Mass Spectrometry Graphs Showing the Tryptic Peptide QVLSVLSQVS of the Diaminobutyrate–2-oxoglutarate Transaminase Protein (*All0396*) at Given Retention Times (X Axis) and Intensities (Y Axis), for the Four Schizokinen Operon Mutant Strains and the Wild Type, Exposed to BG-11 Media with and without Ferric Ammonium Citrate. The retention time predicted on the scheduled transition list for this peptide was 30.4 minutes. Intensities shown (maximum values of y axes), WT BG-11 & BG-11 (-Fe), FC BG-11 & BG-11 (-Fe), FC-*all0395* BG-11 & BG-11 (-Fe): $1.05e^4$, $1.7e^4$, 7467, $2.3e^4$, $3.4e^4$, 1894.

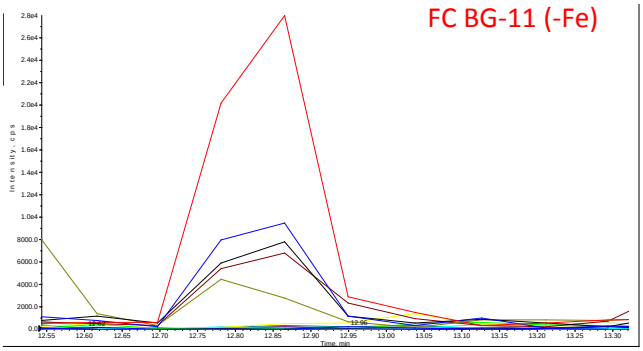

WT BG-11

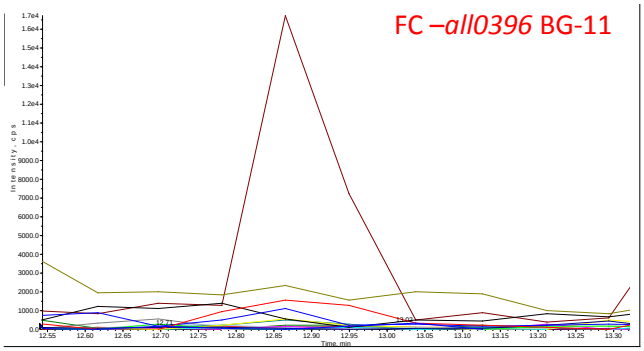

WT BG-11 (-Fe)

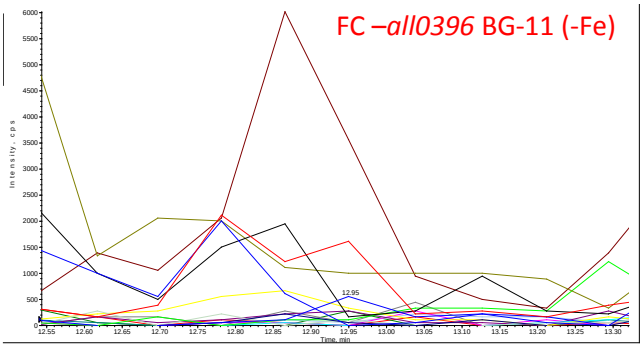

KO BG-11

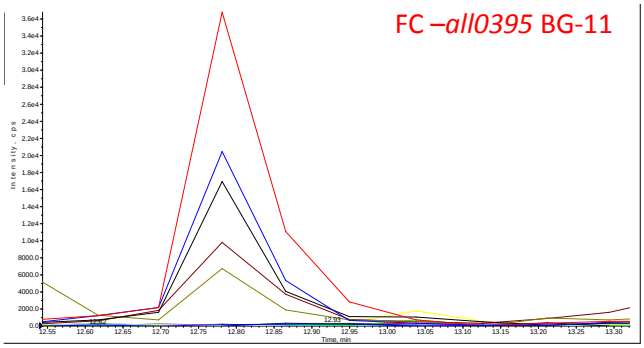

KO BG-11 (-Fe)


FC BG-11


FC BG-11 (-Fe)


FC –all0396 BG-11


FC –all0396 BG-11 (-Fe)


FC –all0395 BG-11

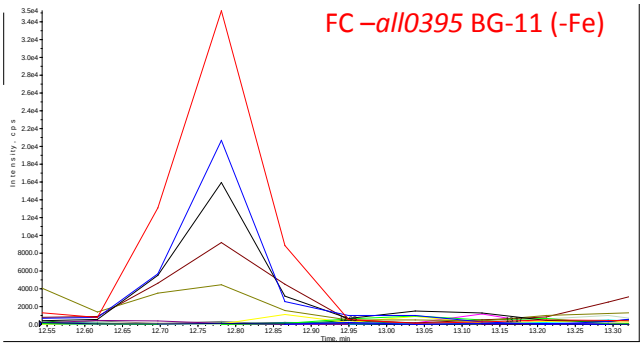
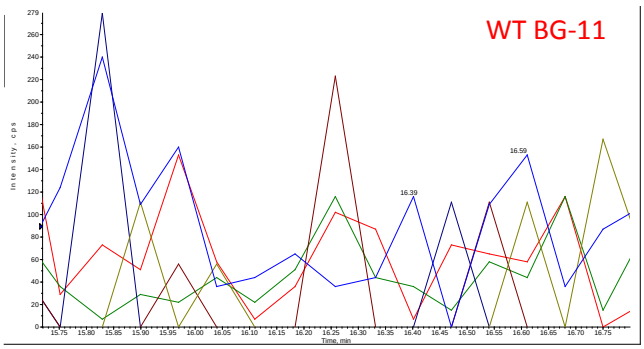
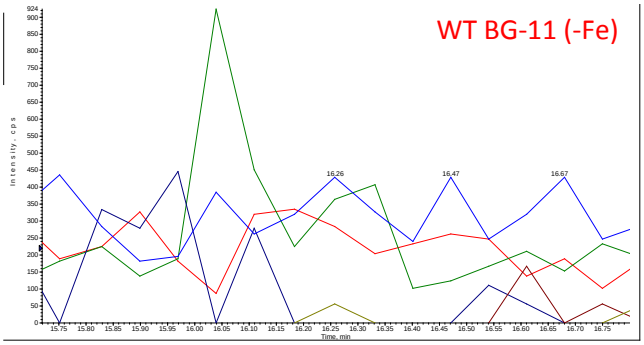
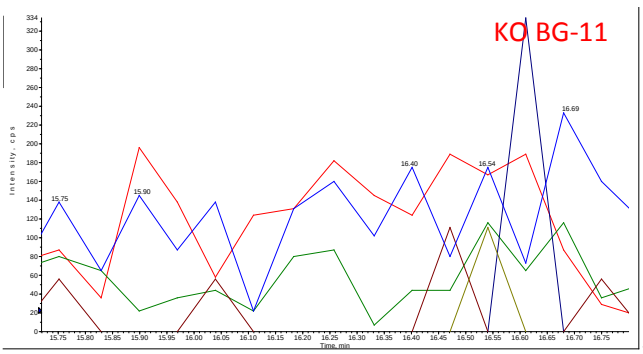
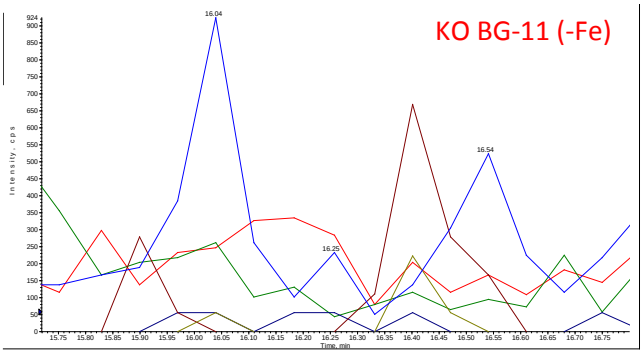

FC –all0395 BG-11 (-Fe)

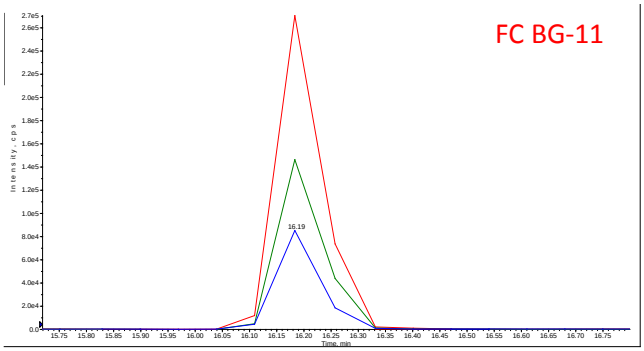
Figure 19: Aligned Multiple Reaction Monitoring Mass Spectrometry Graphs Showing the Tryptic Peptide AIGGSLPLSVVLYNK of the Diaminobutyrate–2-oxoglutarate Transaminase Protein (*All0396*) at Given Retention Times (X Axis) and Intensities (Y Axis), for the Four Schizokinen Operon Mutant Strains and the Wild Type, Exposed to BG-11 Media with and without Ferric Ammonium Citrate. The retention time predicted on the scheduled transition list for this peptide was 12.8 minutes. Intensities shown (maximum values of y axes), WT BG-11 & BG-11 (-Fe), FC BG-11 & BG-11 (-Fe), FC-*all0395* BG-11 & BG-11 (-Fe): $1.3e^4$, $4e^4$, $1.5e^4$, $2.8e^4$, $3.6e^4$, $3.5e^4$.

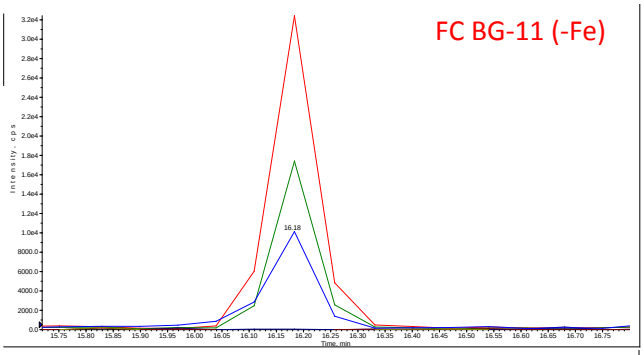

WT BG-11
16.39
16.59

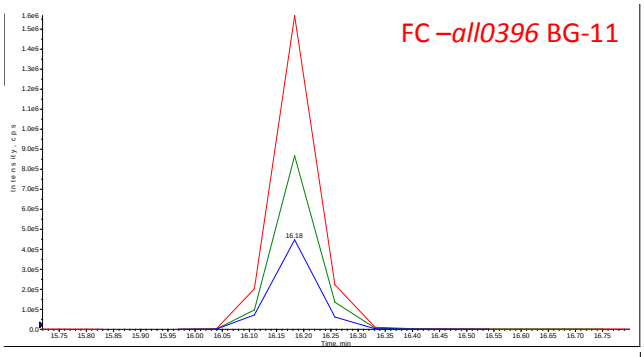

WT BG-11 (-Fe)
16.26
16.47
16.67

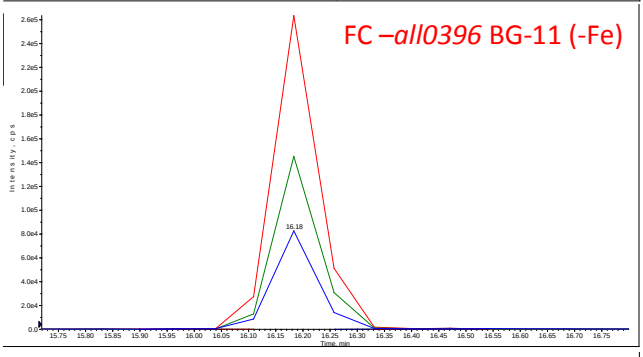

KO BG-11
15.75
15.90
16.40
16.54
16.69

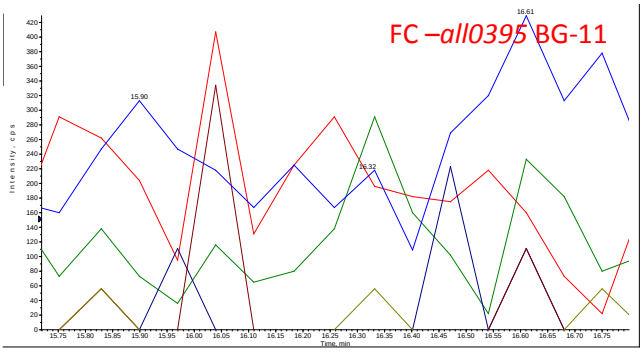

KO BG-11 (-Fe)
16.04
16.25
16.54


FC BG-11
16.19


FC BG-11 (-Fe)
16.18


FC –all0396 BG-11
16.18


FC –all0396 BG-11 (-Fe)
16.18


FC –all0395 BG-11
15.90
16.32
16.61

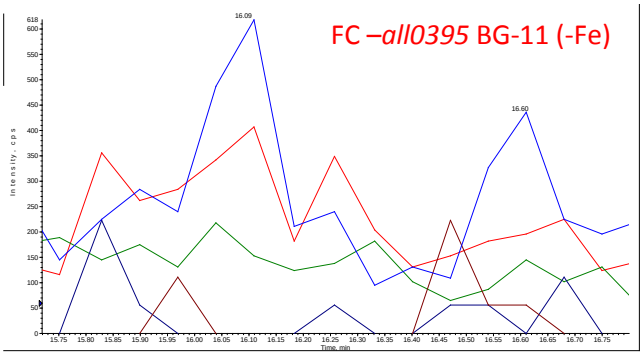
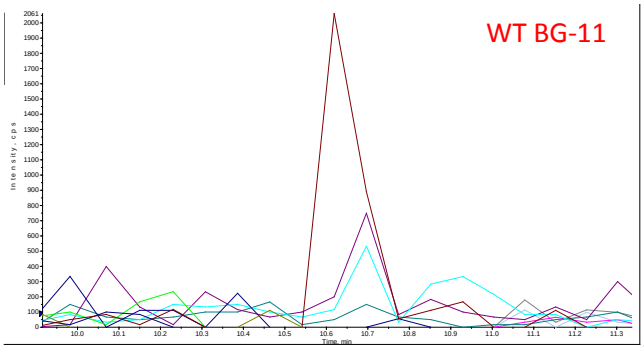
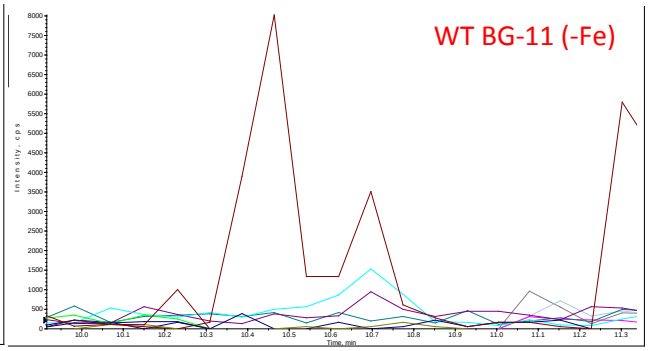
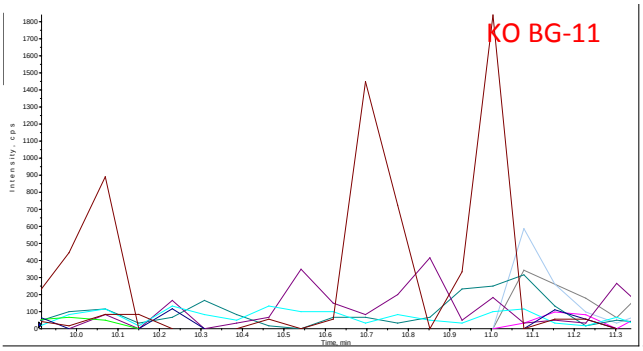
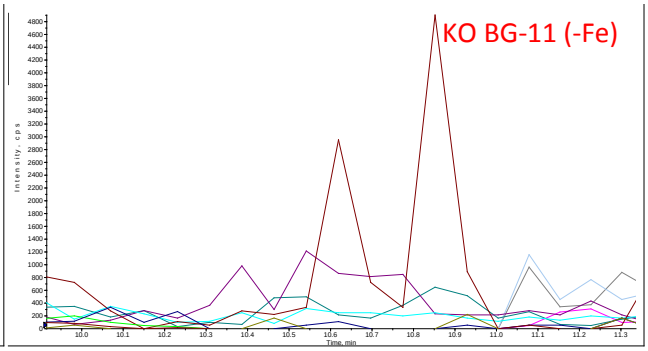

FC –all0395 BG-11 (-Fe)
16.09
16.60

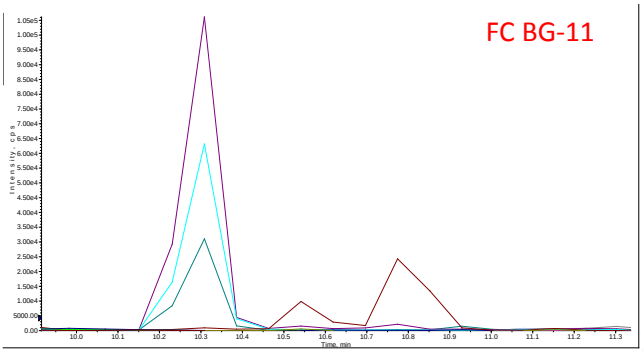
Figure 20: Aligned Multiple Reaction Monitoring Mass Spectrometry Graphs Showing the Tryptic Peptide DFVDDVNISR of the Schizokinen Siderophore Synthase Protein (*All0390*) at Given Retention Times (X Axis) and Intensities (Y Axis), for the Four Schizokinen Operon Mutant Strains and the Wild Type, Exposed to BG-11 Media with and without Ferric Ammonium Citrate. The retention time predicted on the scheduled transition list for this peptide was 16.8 minutes. Intensities shown (maximum values of y axes), FC BG-11 – FC-*all0396* BG-11 (-Fe): $2.7e^5$, $3.2e^4$, $1.6e^6$, $2.6e^5$.

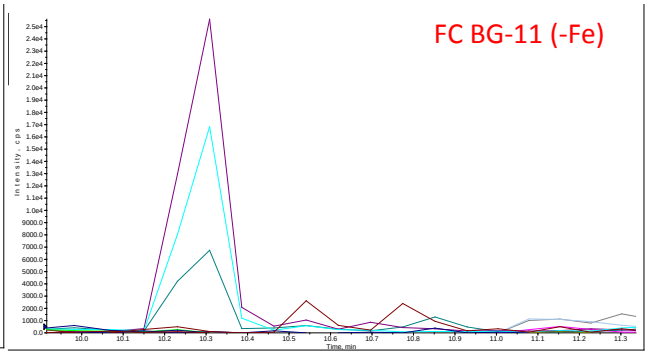

WT BG-11

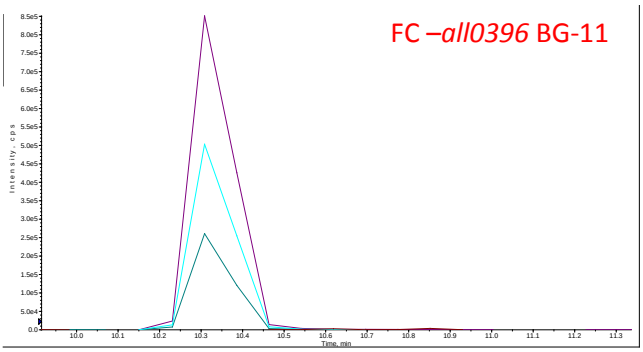

WT BG-11 (-Fe)

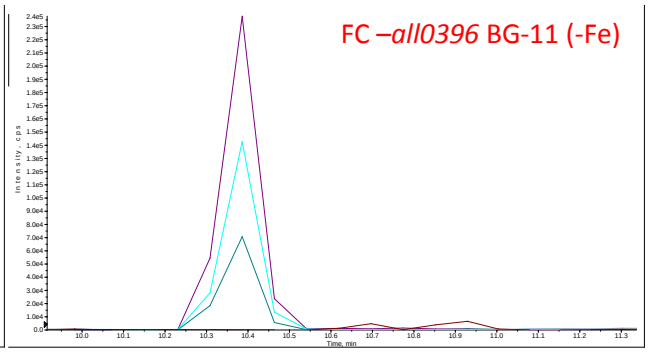

KO BG-11

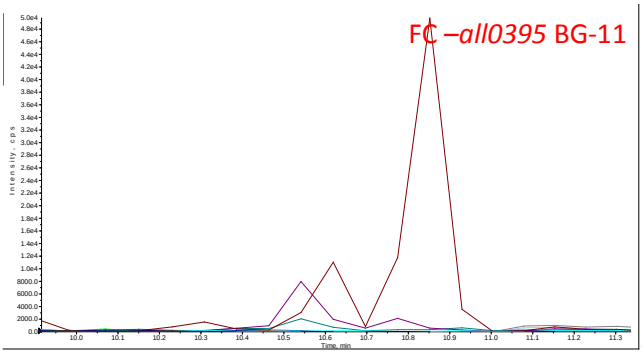

KO BG-11 (-Fe)


FC BG-11


FC BG-11 (-Fe)


FC –*all0396* BG-11


FC –*all0396* BG-11 (-Fe)


FC –*all0395* BG-11

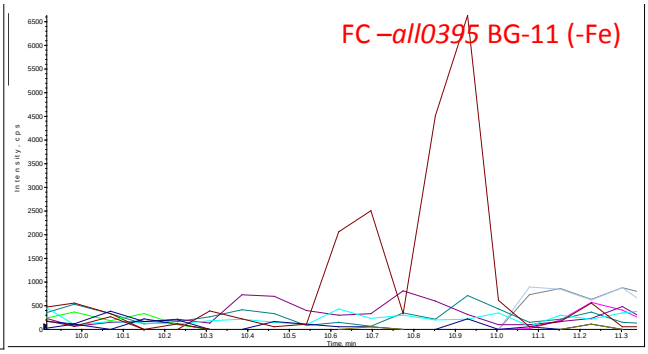

FC –*all0395* BG-11 (-Fe)

Figure 21: Aligned Multiple Reaction Monitoring Mass Spectrometry Graphs Showing the Tryptic Peptide ETILSYQSR of the Schizokinen Siderophore Synthase Protein (*All0390*) at Given Retention Times (X Axis) and Intensities (Y Axis), for the Four Schizokinen Operon Mutant Strains and the Wild Type, Exposed to BG-11 Media with and without Ferric Ammonium Citrate. The retention time predicted on the scheduled transition list for this peptide was 10.8 minutes. Intensities shown (maximum values of y axes), FC BG-11 – FC-*all0396* BG-11 (-Fe): $1.05e^5$, $2.5e^4$, $8.5e^5$, $2.4e^5$.

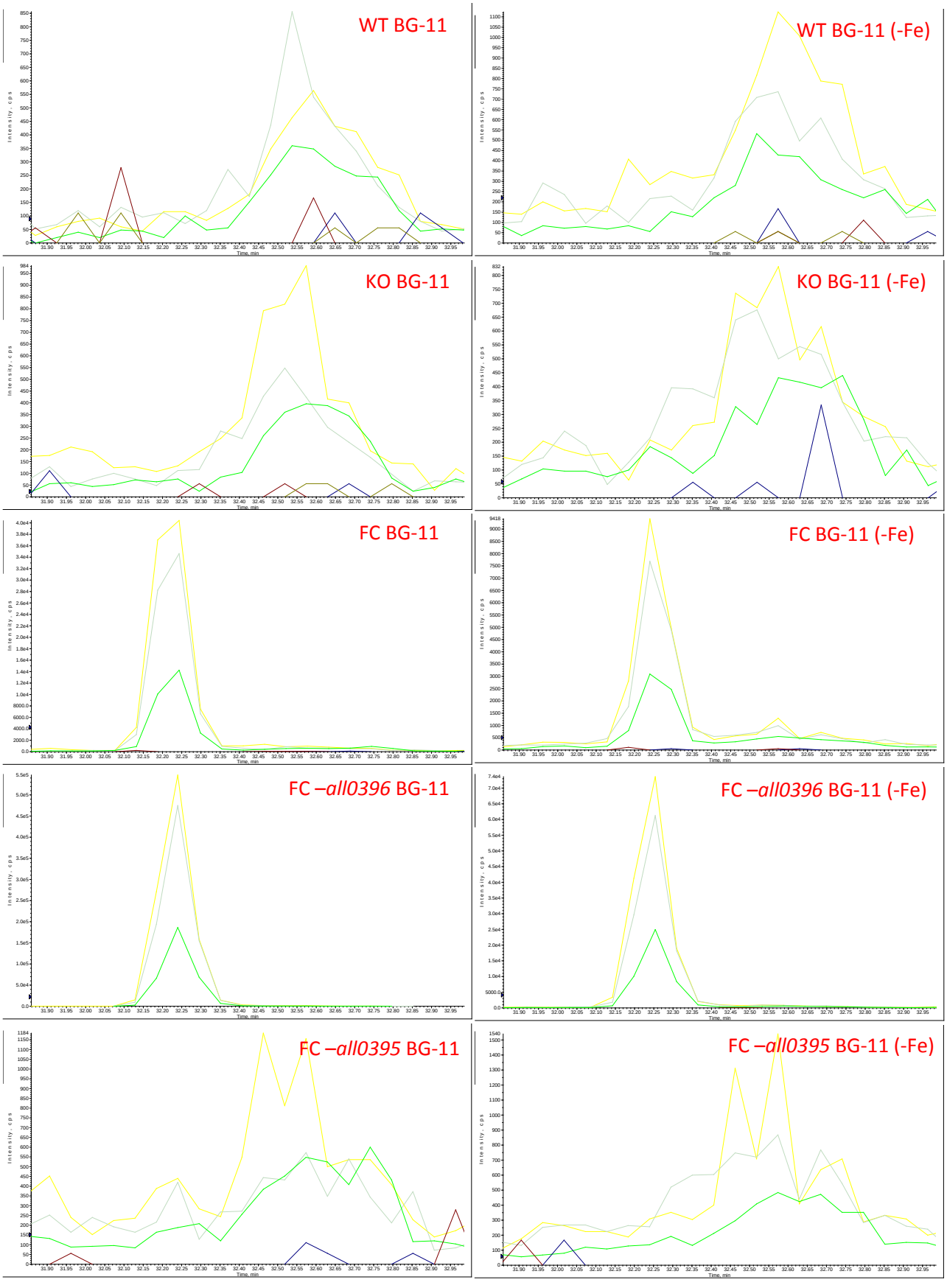
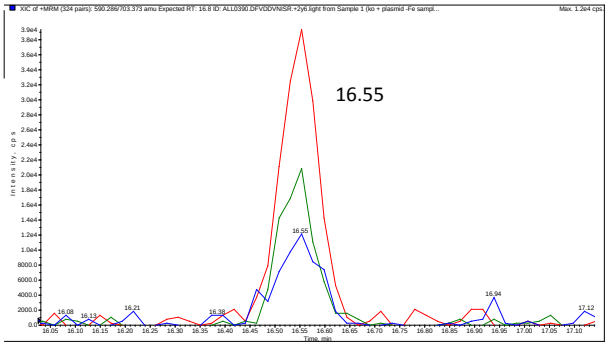
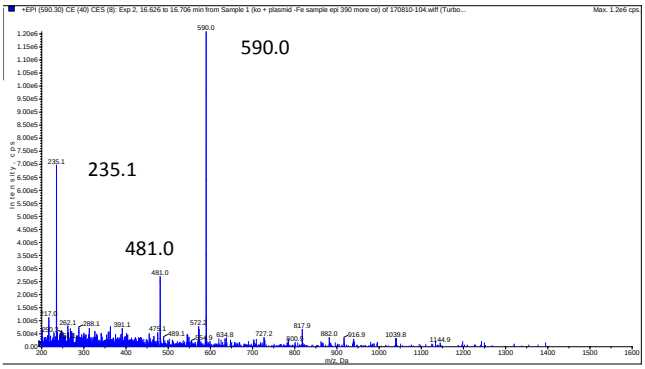

WT BG-11
WT BG-11 (-Fe)
KO BG-11
KO BG-11 (-Fe)
FC BG-11
FC BG-11 (-Fe)
FC –all0396 BG-11
FC –all0396 BG-11 (-Fe)
FC –all0395 BG-11
FC –all0395 BG-11 (-Fe)

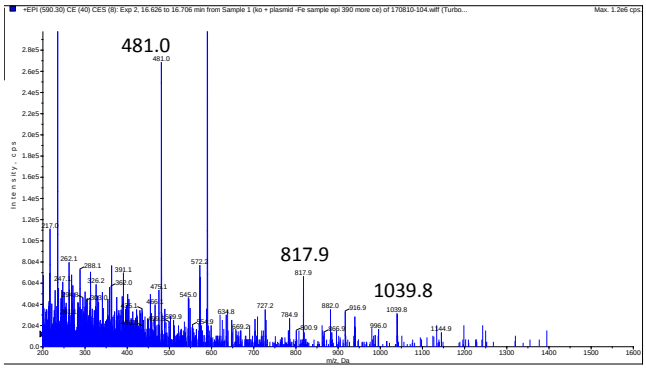
Figure 22: Aligned Multiple Reaction Monitoring Mass Spectrometry Graphs Showing the Tryptic Peptide FELFNLLAPQFTK of the Schizokinen Siderophore Synthase Protein (*All0390*) at Given Retention Times (X Axis) and Intensities (Y Axis), for the Four Schizokinen Operon Mutant Strains and the Wild Type, Exposed to BG-11 Media with and without Ferric Ammonium Citrate. The retention time predicted on the scheduled transition list for this peptide was 32.4 minutes. Intensities shown (maximum values of y axes), FC BG-11 – FC-*all0396* BG-11 (-Fe): $4e^4$, 9418, $5.5e^5$, $7.4e^4$.

Table 7: Details of the Enhanced Product Ion Scans for Tryptic Peptides of the Schizokinen Siderophore Synthase Protein (*All0390*).

Tryptic Peptide Sequence	Parent Ion m/z (Da)	Daughter Ions m/z (Da)	Retention Time (schedule) (mins)	Retention Time (EPI Sample)	Predicted Y Ions m/z (Da)	Presence in EPI Graph
DFVDDVNISR	590.2857	917.4687; 818.4003; 703.3733	16.8	16.55 (see figures 20 and 23)	261.1437 374.22776 488.27069 587.3391 702.36604 817.39298 916.46139 1063.5298 1178.55674	✓ ✓ ✓ ✓ ✓ ✓
ETILSYQSR	548.7853	753.389; 640.3049; 553.2729	10.8	10.71 (see figures 21 and 24)	261.1437 389.20228 552.26561 639.29764 752.3817 865.46576 966.51344 1095.55603	✓ ✓ ✓ ✓ ✓ ✓
FELFNLLAPQFTK	784.4296	1031.588; 804.4614; 620.3402	32.4	32.36 (see figures 22 and 25)	247.1532 394.22161 522.28019 619.33295 690.37006 803.45412 916.53818 1030.58111 1177.64952 1290.73358 1419.77617 1566.84458	✓ ✓ ✓ ✓ ✓ ✓







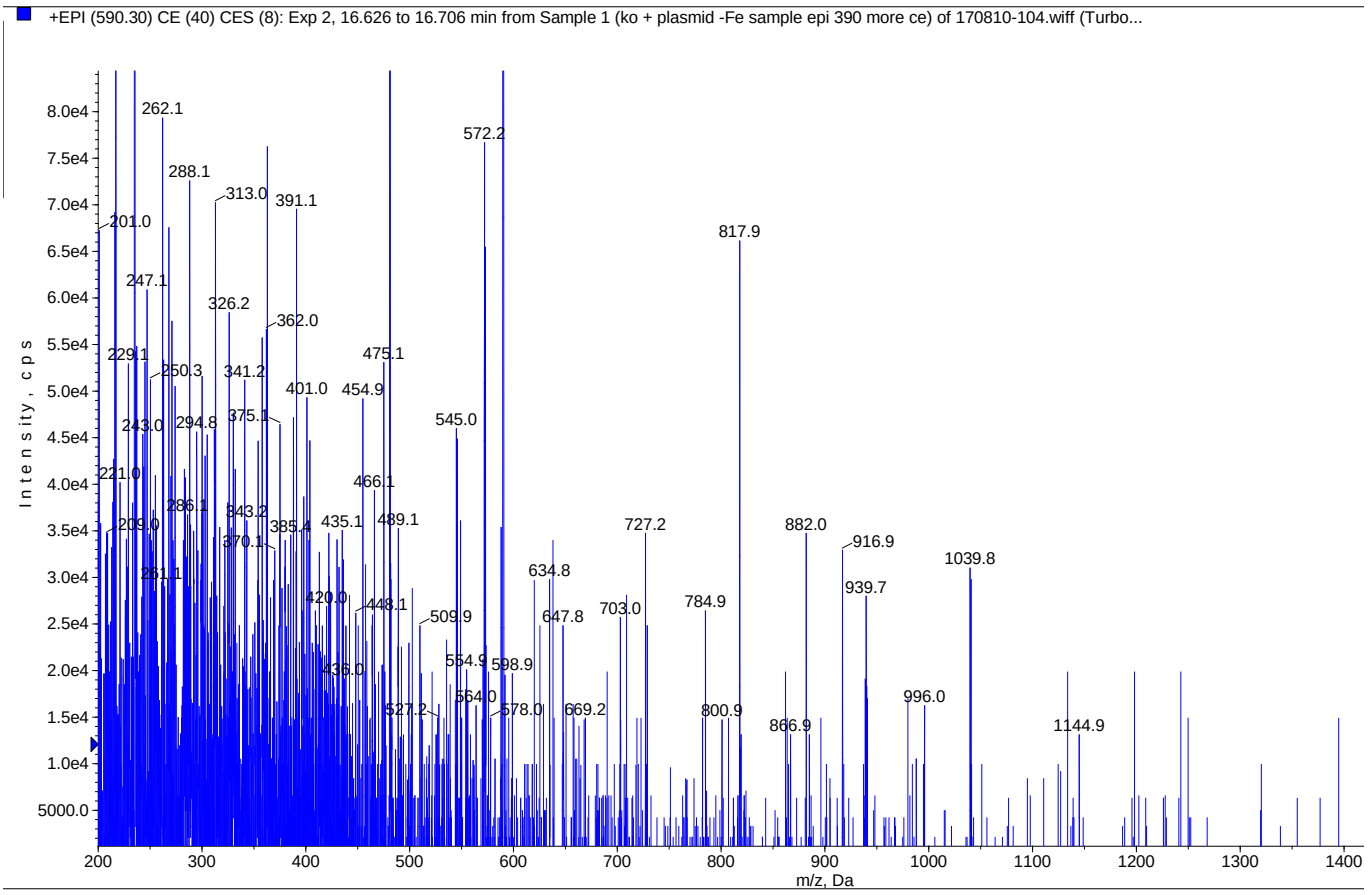
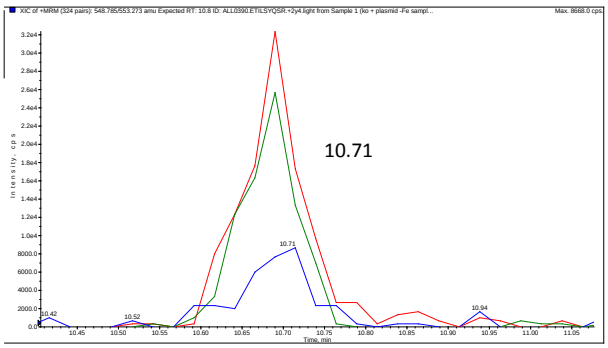
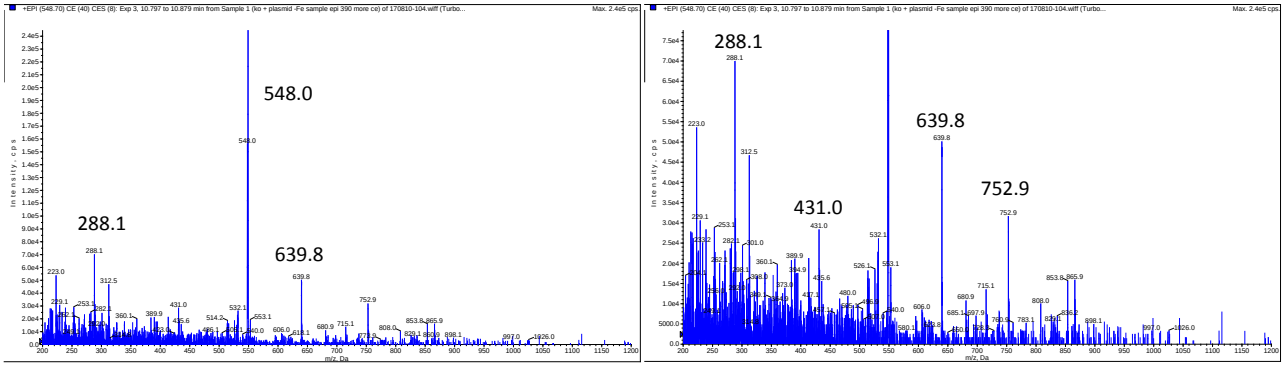


Figure 23: Enhanced Product Ion Scan Graph Showing the Spectrum of Suspected Tryptic Peptide DFVDDVNISR from the Schizokinen Siderophore Synthase Protein (*All0390*). The retention time predicted on the scheduled transition list for this peptide was 16.8 minutes; the MRM graph image above shows an actual retention time of 16.55 minutes in the sample at the time of the EPI – the full complement strain.





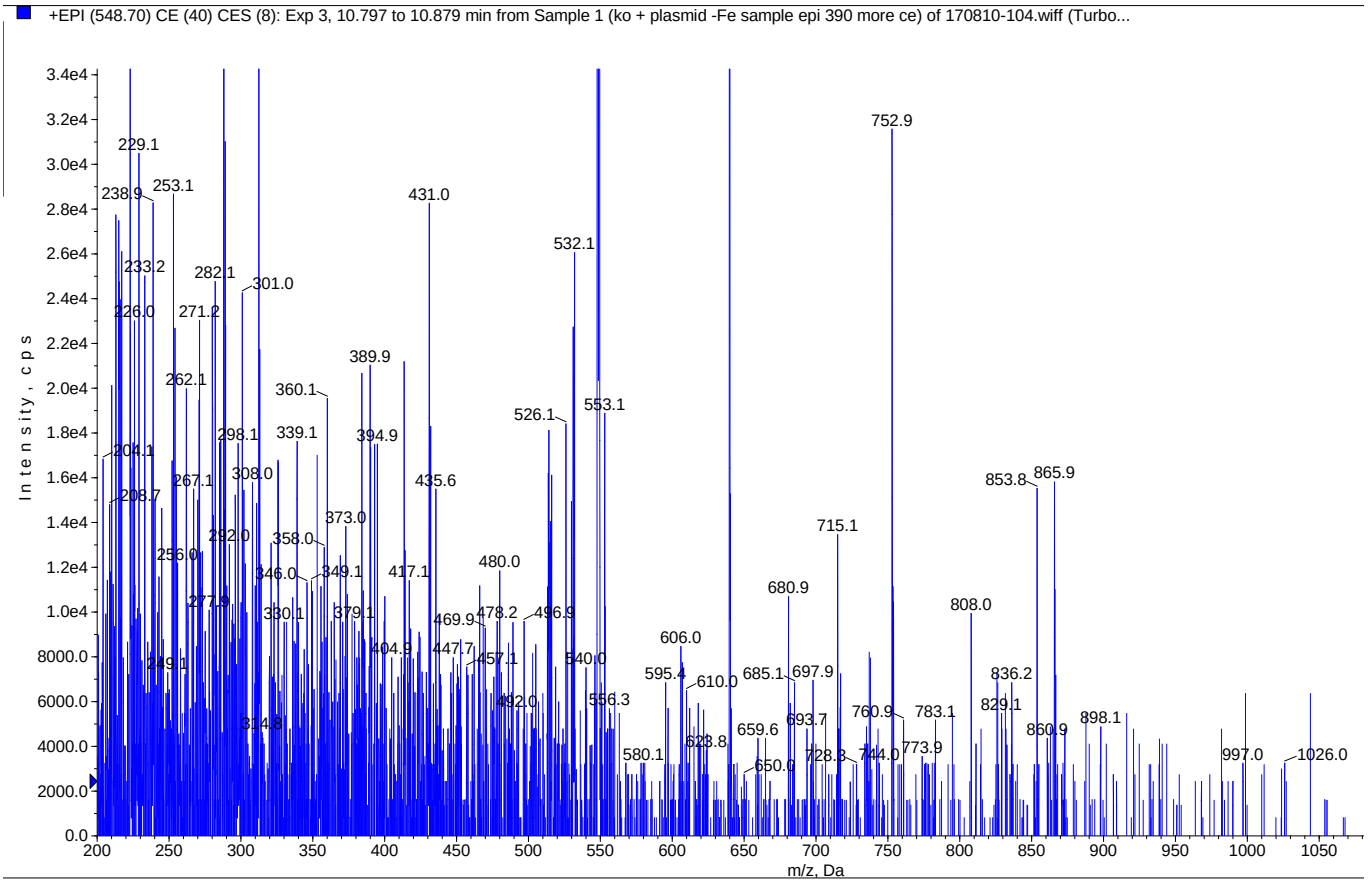
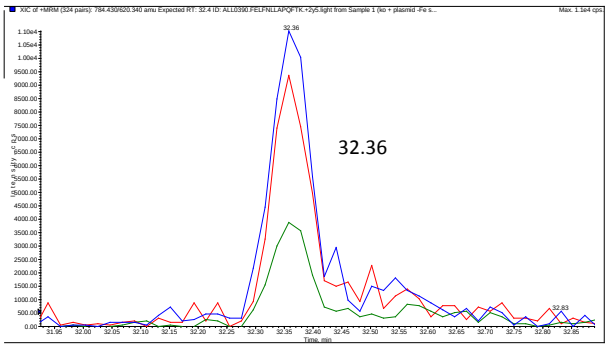
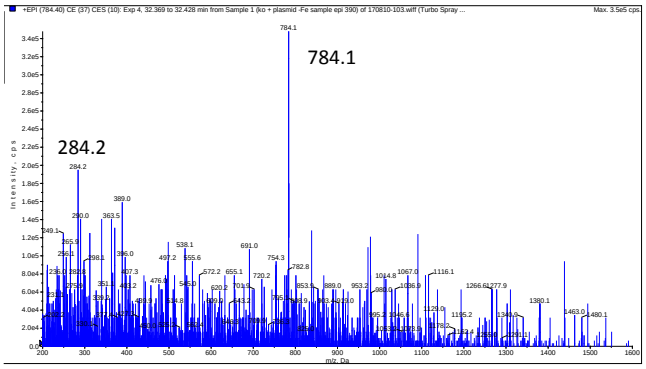


Figure 24: Enhanced Product Ion Scan Graph Showing the Spectrum of Suspected Tryptic Peptide ETILSYQSR from the Schizokinen Siderophore Synthase Protein (*All0390*). The retention time predicted on the scheduled transition list for this peptide was 10.8 minutes; the MRM graph image above shows an actual retention time of 10.71 minutes in the sample at the time of the EPI – the full complement strain.





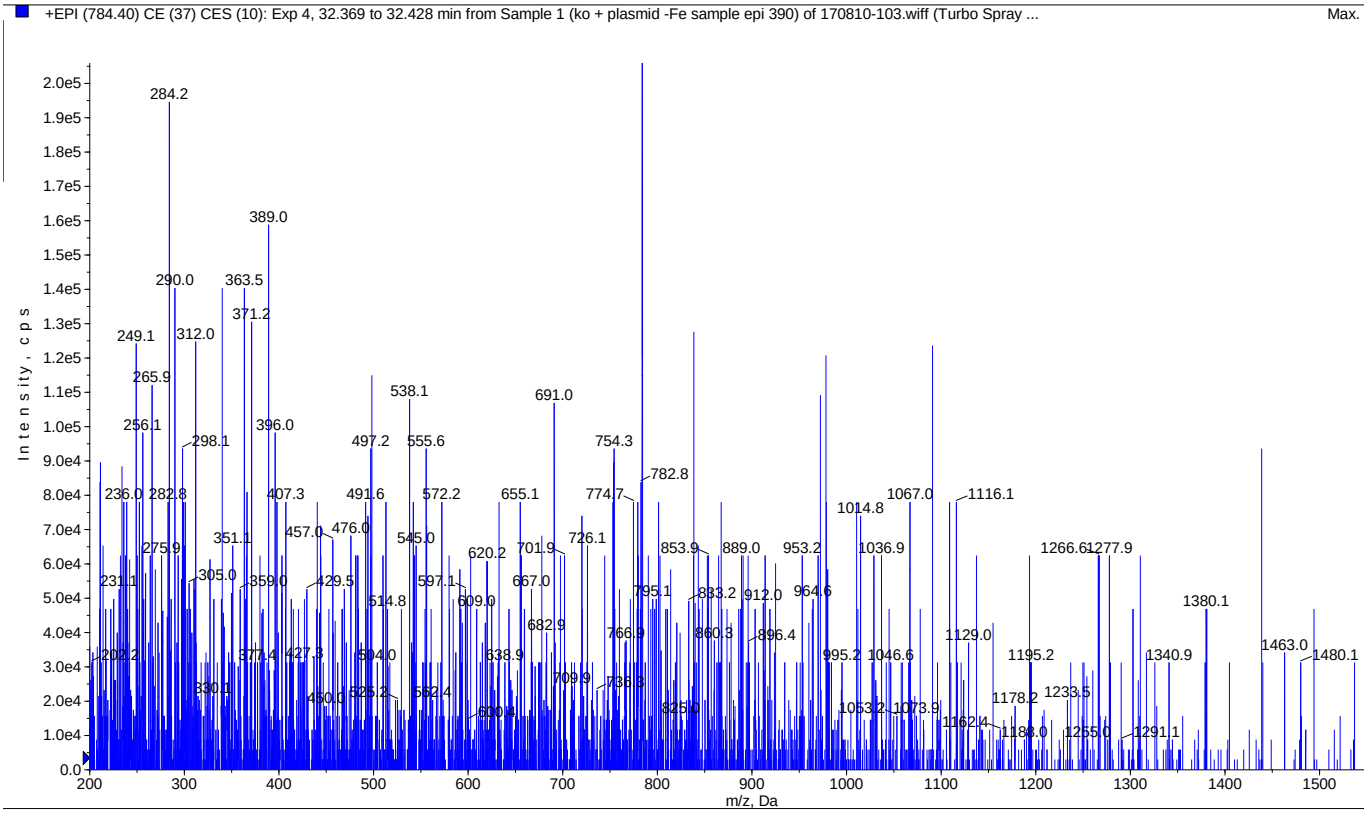


Figure 25: Enhanced Product Ion Scan Graph Showing the Spectrum of Suspected Tryptic Peptide FELFNLLAPQFTK from the Schizokinen Siderophore Synthase Protein (*All0390*). The retention time predicted on the scheduled transition list for this peptide was 32.4 minutes; the MRM graph image above shows an actual retention time of 32.36 minutes in the sample at the time of the EPI – the full complement strain.

Discussion

The results of the various phenotypic and proteomic tests shed light on the importance of the proposed schizokinen operon, as well as strong evidence for its function in iron metabolism. Questions remain over the causes of the discrepancy between the growth performances of the wild type and full complement strains under iron-limited conditions, especially in light of mass spectrometry data; whilst the roles of other siderophores, as well as the relative importance of other iron uptake mechanisms in *Anabaena* 7120 particularly in applicable environmental and agricultural settings, are exposed as further research areas of great interest.

The Importance of the Schizokinen Operon for Growth in Iron-Limited Conditions

As shown most clearly by maximal growth rates and maximum absorbance values achieved at plateau – mu and A parameters respectively – growths of *Anabaena* 7120 cultures were severely impaired in the knock-out mutant strain compared to the wild type in iron-deficient media (media types 2 and 3 - see table 2). This strongly suggests that the expression of genes *All0390-All0396* – encoding schizokinen synthesis enzymes – and therefore the synthesis of the schizokinen siderophore itself are important for rapid cell growth in iron-limited conditions. This is consistent with previous observations on the importance of this specific mode of iron uptake in *Anabaena* 7120 – individual knock-out mutants of genes expressing the Ton-B dependent outer membrane schizokinen transporters SchT (*Alr0397*) [18; 8] and IutA2 (*Alr2581*) [23; 8] are known to exhibit iron-deficiency phenotypes and growth impairments, as well as knock-out mutants of *TonB3*, *ExB3/D3*, and *Fhu* which encode other factors involved in the schizokinen transport system [27]. *Alr0397* (SchT) has also been found to be upregulated [2; 18], and the rate of schizokinen uptake increase [22], under iron-limited conditions, supporting this explanation.

Given the widespread interest in applications of *Anabaena*, the question arises over the ecological relevance of these well-supported findings of the importance of the schizokinen-iron-uptake system in laboratory settings – especially considering the forms of ferric iron typically available in natural freshwater environments [22]. In these settings, studies have shown over 99 per cent of ferric iron to be occupied by organic ligands [33], with only small, nanomolar-range concentrations of iron hydroxides being the prominent inorganic species [30]. Hence a consideration the importance of mechanisms of iron uptake other than the secretion of siderophores – i.e. the uptake of exogenous siderophores from other species, particularly in comparison with the former under iron-limited conditions would be informative on this issue. This has been tested under standard conditions before – for example comparing the uptake of the desferrioxamine B (an exogenous siderophore)-iron complex with schizokinen-iron, where the latter was found to be more efficient [22]. Growth performance findings regarding wild type and knock-out mutant strains in this study are also consistent with the hypothesis that in high-density cultures, typical of laboratory settings and particularly when growth assays are conducted in low-volume apparati – such as 6-well plates – release of schizokinen is of much greater importance than inorganic iron uptake or the uptake of exogenously-chelated iron in iron-limited conditions [22]. For cultures at lower densities, such as may be more relevant in native or agricultural settings, the uptake and reduction of inorganic ferric hydroxides, as well as uptake of exogenously-chelated iron (mechanisms which have not been found

to be upregulated in iron-limited conditions under laboratory settings) could be of greater importance for growth [22].

The Failure of Full Schizokinen Operon Complementation to Restore Growth Performance

In the growth assays conducted in 6-well plates, the full complement strain did not show a growth performance in any way similar to or better to that of the wild type – as clearly shown in the mu and A parameter comparisons results. Instead the full complement strain, despite containing the introduced RSF1010 plasmid with the full schizokinen operon (*All0390-All0396*), had growth more similar to that of the knock-out mutant which lacked the operon entirely. As to why the full complement strain appeared to have a defunct schizokinen iron uptake system, or whether the growth performance was due to another factor, would require further investigation. Given the evidence of gene expression control by *trans*-regulatory elements – Fur proteins [9; 15], it seems unlikely but at least still a possibility that the discrepancy could be due to *cis*-regulation. However, for *All0394*, *All0396*, and *All0390*, mass spectrometry multiple reaction monitoring graphs data, and particularly in the case of *All0390* given an enhanced product ion scan, this was shown to not be the case, with expression detected. Furthermore, mass spectrometry analysis has not shown consistent evidence for reduced expression levels of these genes in the full complement compared to detected levels in other strains (figures 16-22 – intensities of peaks detailed in legends). Further mass spectrometry analyses, including those focussing on transient metabolites in the synthesis pathway of schizokinen, and levels of schizokinen itself, could provide further insights into this problem. After all, given the predicted synthesis pathway [15], lack of expression of a single relevant gene would disable the schizokinen iron uptake system.

Conclusion

Anabaena 7120 loci *All0390-All0396* encode proteins with highly important roles in iron metabolism, as shown here by the significantly negative effects of whole-operon knock out on growth performance in iron-limited conditions in a laboratory setting. Together with *Alr0397*, which encodes the schizokinen transporter [18], these genes are regulated by ferric uptake regulator proteins and respond with elevated expression to such conditions [15], making them arguably forming the most important iron uptake mechanism in this cyanobacterial subspecies. However, understanding the diverse modes of iron uptake and their relative importance in iron metabolism of *Anabaena* 7120 required a multifaceted approach, as well as isolation of and experimentation on other possible siderophores; and quantitative investigation into the expression and regulation of expression of such genes.

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RELEVANT WEBSITES:

Grofit R Package: https://cran.r-project.org/web/packages/grofit/grofit.pdf

ProSight PTM® Ion Predictor: https://prosightptm.northwestern.edu/ionpredictor/

Proteins of Interest Sequences, Mass Spec, (NCBI Website):
https://www.ncbi.nlm.nih.gov/protein/17129740; https://www.ncbi.nlm.nih.gov/protein/17129741;
https://www.ncbi.nlm.nih.gov/protein/17129737; https://www.ncbi.nlm.nih.gov/protein/17129738;
https://www.ncbi.nlm.nih.gov/protein/17129739; https://www.ncbi.nlm.nih.gov/protein/17129735;
https://www.ncbi.nlm.nih.gov/protein/17129742;
https://www.ncbi.nlm.nih.gov/protein/BAB75700.1;
https://www.ncbi.nlm.nih.gov/protein/BAB77890.1;
https://www.ncbi.nlm.nih.gov/protein/17131743

Appendix A: Materials and Methods, Further Details

Preliminary Cyanobacterial Growth Method Growth Assays

Appendix Table A(i): Treatment Groups of the Preliminary Cyanobacterial Growth Method Growth Assays – Conducted using Flasks, 6-Well Plates, and the Multi-Cultivator 1000

Apparatus used	Species	Volume of Culture	Carbon Dioxide Concentration	Strain	Media	Measurement Method (Dilution)*	Sample size (Replicates)	Total Number of Treatment Groups*	Total Number of Data Sets
Flasks	*Anabaena* 7120	25 ml	Atmospheric	Wild Type	BG-11	Diluted (10X)	3	4	12
						Undiluted	3		
			Elevated (1%)	Wild Type		Diluted (10X)	3		
						Undiluted	3		
	Synechocystis 6803		Atmospheric	Wild Type		Diluted (10X)	3		
						Undiluted	3		
			Elevated (1%)	Wild Type		Diluted (10X)	3		
						Undiluted	3		
6-well plates	*Anabaena* 7120	3 ml	Elevated (1%)	Wild Type		Diluted (10X)	3	2	6
						Undiluted	3		
	Synechocystis 6803		Elevated (1%)	Wild Type		Diluted (10X)	3		
						Undiluted	3		
MC1000	*Anabaena* 7120	50 ml	Atmospheric	Wild Type		-	3	2	6
	Synechocystis 6803		Atmospheric	Wild Type			3		

* Optical Density at 720nm was measured throughout as an assessment of cyanobacterial growth. For flasks, undiluted measurements were taken by pipetting 200 µl culture into 96-well plates, whilst for 6-well plate cultures undiluted measurements were made directly from the 6-well plates. In both cases, 10-times-diluted measurements were made by diluting 20 µl culture in 180 µl BG-11 media in 96-well plates. Flask and 6-well plate data was collected daily and measured using an Infinite® 200 PRO NanoQuant Multimode Microplate Reader (Tecan), and MC1000 optical density measurements were taken every 30 minutes.

Preliminary Cyanobacterial Growth Method Growth Assays

Prior to the *Anabaena* schizokinen mutant strains growth assays, a set of week-long preliminary experiments were performed with the in order to assess the most suitable methods for culturing *Anabaena* 7120 (as well as another cyanobacterial species, *Synechocystis* 6803) in growth assays, with regards to growing conditions, and apparatus used. BG-11 media (see below) was used throughout. Flasks, 6-well plates, and the Multi-Cultivator® 1000 incubator (MC1000) (Photon Systems Instruments) were tested, along with conditions of atmospheric and elevated carbon dioxide, and different methods for collecting growth by using optical density measurements. Treatment groups for each of the different apparati (flasks, 6-well plates, and the MC1000) are detailed in appendix table A(i). Based on the results of these preliminary growth assays, the quality of data fits to growth curve models, and considerations of space constraints, 6-well plates and the MC1000 were chosen as the most suitable apparati for the *Anabaena* Schizokinen mutant strains growth assays; and two weeks was decided on as the duration of the assays.

The most useful Grofit model for fitting data was the logistic model (appendix tables B(ii-ix) appendix figures B(i-v)), indicating classic growth curves consisting of lag, log, and plateau phases [7]. Regarding apparati, the MC1000 produced the data with the least variation in the fitting of growth curve models (appendix figures B(vi-x)). 6-well plates were useful for conducting growth assays with a large number of treatment groups and replicates, considering space availability; however evaporation from wells could be problematic and raise concerns of contamination between samples, so great care had to be taken when taking measurements. For the *Anabaena* schizokinen mutant strains growth assays, the decision was taken to conduct 6-well plate-based assays for all treatment groups initially, and then follow this up with repeated MC1000-based assays for a smaller number of treatment groups.

Phenotypic Evidence: *Anabaena* Schizokinen Mutant Strains Growth Assays

Assembling and Confirming Mutant Strains – Colony PCRs

Colony Polymerase Chain Reactions to confirm the identity of mutant strains were performed using the Q5® High-Fidelity PCR Kit (New England Biolabs), with a recipe of 5X (4 µl) Q5® reaction buffer, 10 µM (1 µl) of each primer, 2 µl DNA template, 0.2 µl Q5® DNA polymerase, and nuclease-free water making up a 20 µl total reaction volume. Template DNA was prepared by heating agar-plate cells in nuclease-free water at 90°C for 10 minutes, before five freeze-thaw cycles at -80°C for four minutes and 60°C for two minutes. The PCR programme consisted of 98°C for 30 seconds followed by 35 cycles of 98°C for 10 seconds, 65°C for 20 seconds, and 72°C, with a final stage of 72°C for 2 minutes.

Phenotypic Evidence: *Anabaena* Schizokinen Mutant Strains Growth Assays **& Preliminary Cyanobacterial Growth Method** Growth Assays

Statistical Analysis of Growth Assay Data

Grofit Package in R – Worked Example

```
> local({pkg <- select.list(sort(.packages(all.available = TRUE)),graphics=TRUE)
+ if(nchar(pkg)) library(pkg, character.only=TRUE)}) # load grofit package, after installation and setting CRAN mirror.
```

2 datasheets are set up as .csv files on excel and imported correctly into R – one for the time data [here called "time"] and one for the growth data [here called "data"]:

```
> time <- read.csv (file.choose())
> time
```

Sorted the data sheet out on excel csv

The time data is in this format:

time value 1	time value 2	time value 3	time value 4	time value 5	…	time value n
time value 1	time value 2	time value 3	time value 4	time value 5	…	time value n
time value 1	time value 2	time value 3	time value 4	time value 5	…	time value n
…	…	…	…	…	…	…

m+1 rows in this way, were m = number of data sets; n = number of time values

```
> data <- read.csv (file.choose())
> data
```

Sorted the data sheet out on excel csv

The growth data is in this format:

Experiment	Details/ID	Concentration				
Experiment 1	WTBG	0	value 1_1	value 2_1	…	value p_1
Experiment2	WTBG-Fe	0	value 1_2	value 2_2	…	value p_2
…	…	…	…	…	…	…
Experiment q	C9BG-Fe+Che	50	value 1_q	value 2_q	…	value p_q

Where q = number of datasets; p = number of growth values in each dataset. Regarding both the time data, and the growth data, q = m and p = n.

```
> myOptions <- grofit.control(fit.opt = "m", interactive = FALSE, model.type=c("logistic" , "gompertz" , "richards" , "gompertz.exp")) # This will allow the grofit package to state which of the four models fits the data best in each case (for each dataset / replicate of a treatment group).
> myOptionsLogistic <- grofit.control(fit.opt = "m", interactive = FALSE, model.type=c("logistic"))
> myOptionsGompertz <- grofit.control(fit.opt = "m", interactive = FALSE, model.type=c("gompertz"))
> myOptionsRichards <- grofit.control(fit.opt = "m", interactive = FALSE, model.type=c("richards"))
> myOptionsGompertzExp <- grofit.control(fit.opt = "m", interactive = FALSE, >
model.type=c("gompertz.exp"))
```

This sets up various options of model fits, after testing all the datasets with "myOption" to see which model types fit which datasets best

```
> modeltestresult <- gcFit(time, data, control = myOptions)
> print(summary(modeltestresult)) #  R shows the results from running the models using myOptions, including which model fits each dataset best. The parameters of lambda, mu, and A will also be shown, as well as the 95% upper and lower confidence interval values for each. Then afterwards, one can repeat the whole process, but doing one dataset at a time, with the preferred model. Model fits can also be plotted, see below.
> plot(modeltestresult) # Plots the model curves
```

Ditto, but for individual model types:

```
> modeltestresult2 <- gcFit(time, data, control = myOptionsLogistic)
> print(summary(modeltestresult2))
> plot(modeltestresult2) # Plots the model curves
> modeltestresult3 <- gcFit(time, data, control = myOptionsGompertz)
> print(summary(modeltestresult3))
> plot(modeltestresult3) # Plots the model curves
> modeltestresult4 <- gcFit(time, data, control = myOptionsRichards)
> print(summary(modeltestresult4))
> plot(modeltestresult4) # Plots the model curves
```

```
> modeltestresult5 <- gcFit(time, data, control = myOptionsGompertzExp)
> print(summary(modeltestresult5))
> plot(modeltestresult5) # Plots the model curves
```

Phenotypic Evidence: Chrome Azurol S Assay

Chrome Azurol S solid media was prepared using the protocol previously described in another study involving *Anabaena* 7120 [18]. 1/10 volume of Chrome Azurol S solution was added to BG-11 liquid media. To make CAS solution, 60.5mg Chrome Azurol S (Sigma-Aldrich) in 50ml deionised water was mixed with 1mM ferric chloride – hexa-hydrate in 10ml 10mM HCl and 72.9mg HDTMA (Hexadecyltrimethylammonium bromide, Sigma-Aldrich) in 40ml deionised water. One percent Bacto agar was finally added before autoclaving.

Proteomic Evidence: Mass Spectrometry of Proteins (more materials are in appendix E)

Protein Extractions

To extract protein, cultures of cells (50-100ml) were pelleted, resuspended with extraction buffer (0.02M Tris-HCl (pH 8.0), 0.001M EDTA, 0.002M DTT) and then shaken with extraction buffer-washed glass beads at 30Hz for two minutes. Cell fragments were then pelleted and removed. Protein concentrations were measured using Biorad® protein assay reagents A and B.

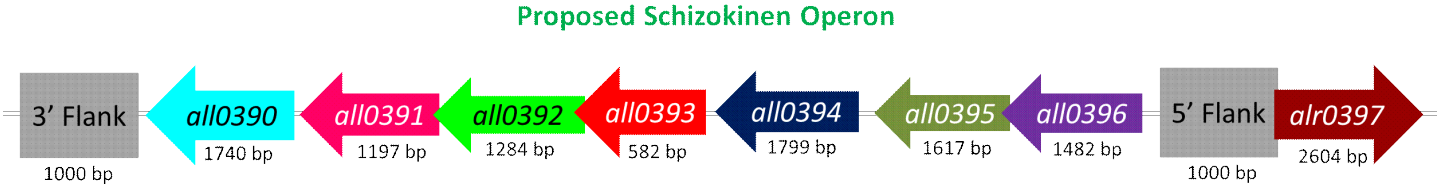
Trypsin Digestions

Protein samples of 3µg/µl were prepared for digestion, using proteomics-grade trypsin (Promega® kit, V5117), after reduction by 1/100 volume 1M DTT and 1/20 volume 1M ammonium bicarbonate (left at 56°C for one hour), and alkylation by 1/10 volume 50mM iodoacetamide (left at 37°C for 30 minutes). The overnight digests were arrested with 1/100 volume 98% formic acid (37°C for 30 minutes), and finally undigested insoluble products were pelleted and removed. Digests were tested via SDS-PAGE.

Mass Spectrometry Apparati

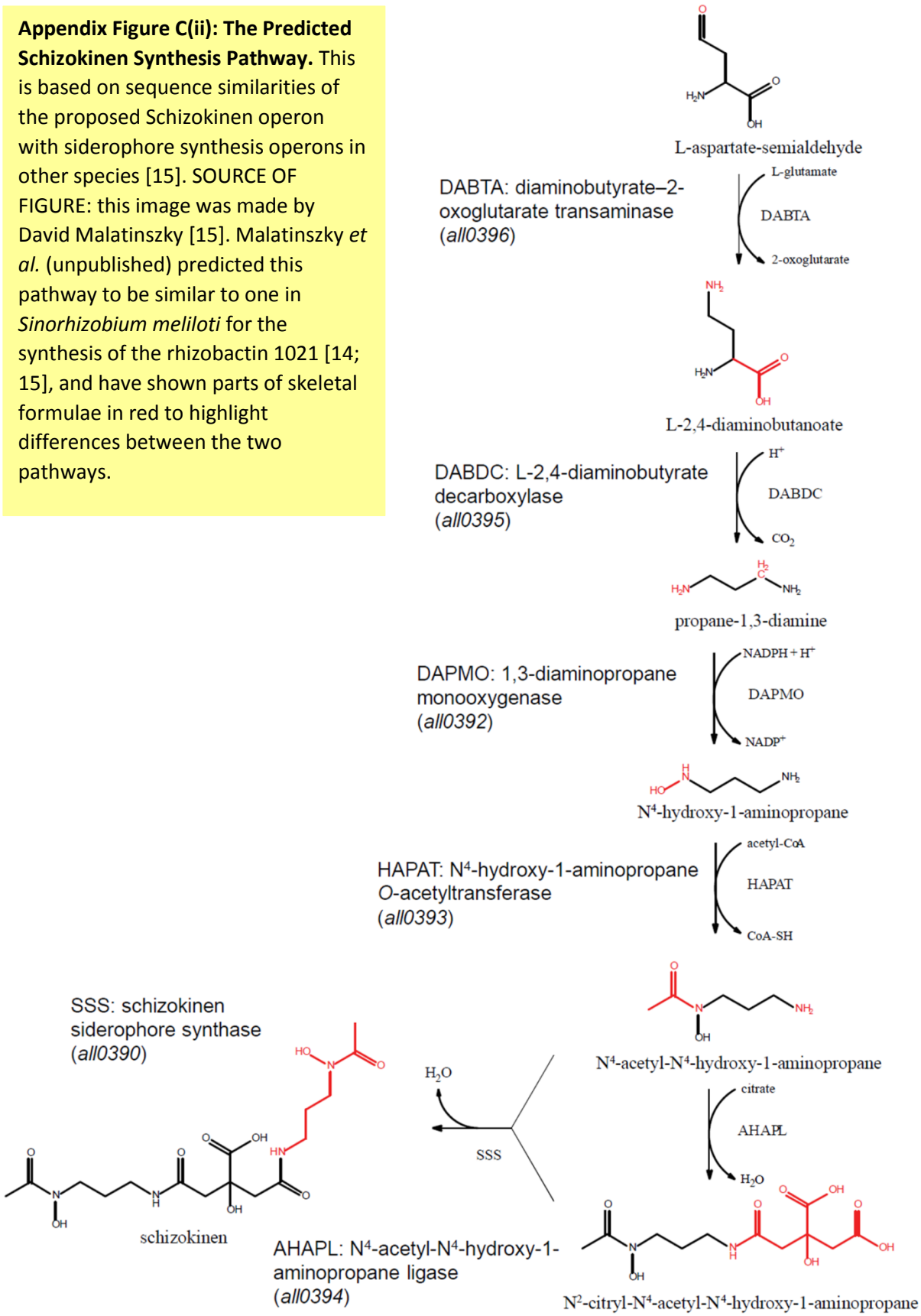
The apparatus used to analyse samples (trypsin-digested protein extractions) was ABSIEX 6500QTrap Mass Spectrometer, working with a Shimadzu UPLC system. The latter was used with an Ion Drive Turbo V source. A Luna C18 column was used to separate peptides, with a flow rate of 250µl/min, and a solvent gradient consisting of 94.9-5.0 per cent water, 5.0-94.9 per cent methyl cyanide, and 0.1-0.1 per cent CHOOH. After two minutes, the flow from the column was directed into the mass spectrometer. 40psi curtain gas, 40psi GSI, and 60psi GS2, Interface heater active, and TEM 500^{o}C were there settings for the mass spectrometer. MRMs were run in “Triple Quadruple” mode and EPIs in “Trap” mode. The typical volume of injection of samples was 20µl.

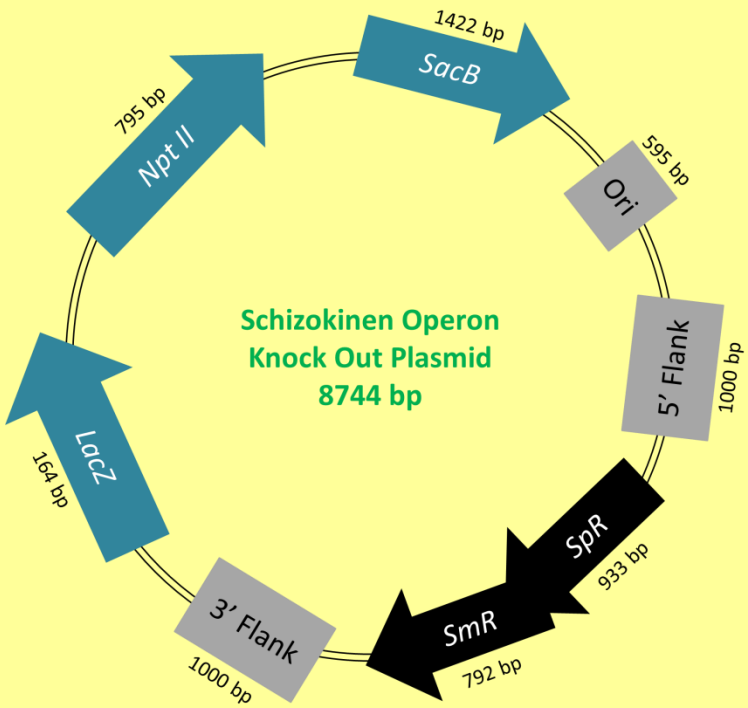
Appendix C: Schizokinen Mutants and Wild Type



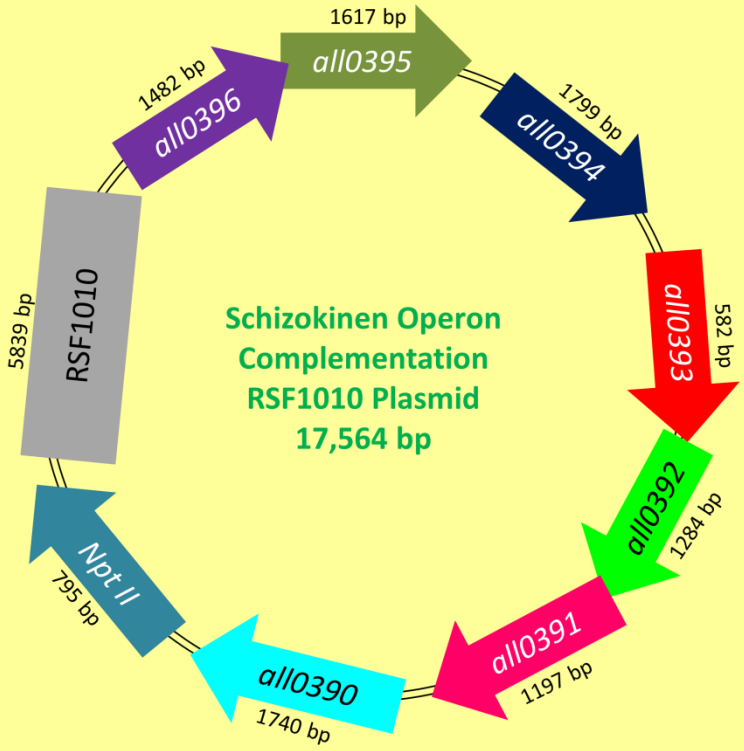
Appendix Figure C(i): The Schizokinen Operon, as proposed previously (adapted from Malatinszky *et al.* unpublished [15]). Genes *all0390* and *all0392-6* are predicted to be involved in the putative schizokinen synthesis pathway [15; 8] (see appendix figure B(ii)), but this has not yet been metabolically characterised fully.

Appendix Figure C(ii): The Predicted Schizokinen Synthesis Pathway. This is based on sequence similarities of the proposed Schizokinen operon with siderophore synthesis operons in other species [15]. SOURCE OF FIGURE: this image was made by David Malatinszky [15]. Malatinszky *et al.* (unpublished) predicted this pathway to be similar to one in *Sinorhizobium meliloti* for the synthesis of the rhizobactin 1021 [14; 15], and have shown parts of skeletal formulae in red to highlight differences between the two pathways.

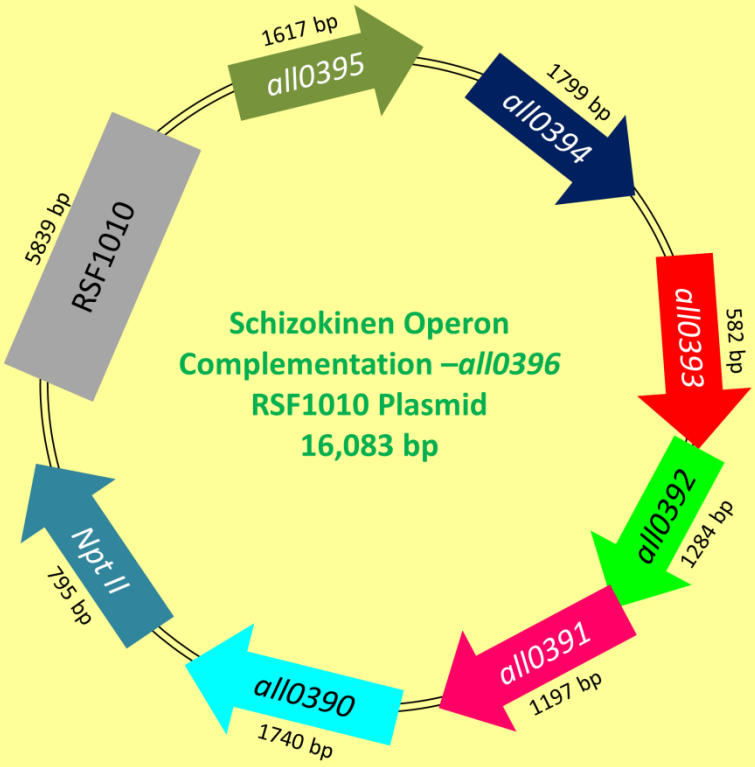


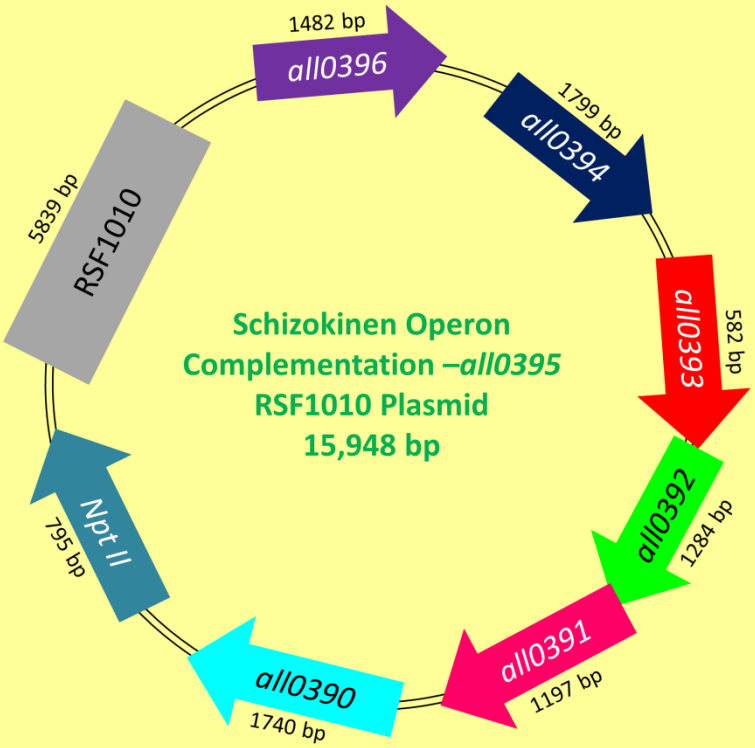


Appendix Figure C(iii): Schizokinen Operon Knock Out Plasmid (see sequence for further detail). This plasmid was used to create the double-recombination schizokinen operon knock-out mutant, by replacing the genes *all0390* – *all0396* and *alr0397* with spectinomycin and streptomycin resistance marker genes. The plasmid contains 5' and 3' flanking sequences for the operon, which reflect surrounding regions of the genes of interest in the genomic sequence [15; 8]. *NptII*: *Neomycin Phosphotransferase II*; *LacZ*: encodes beta-galactosidase; *SacB*: *Levansucrase*; *Ori*: origin of replication. (Adapted from Malatinszky *et al.,* unpublished. [15].)



Appendix Figure C(iv): Schizokinen Operon Complementation RSF1010 Plasmid (see sequence for further detail). This plasmid was used to create the FC–*all0396* complement mutant, via introduction into the knock-out mutant; hence it contains genes *all0390* – *all0396*. *NptII*: *Neomycin Phosphotransferase II*. (Adapted from Malatinszky *et al.,* unpublished [15].)





Appendix Figures C(v & vi): Schizokinen Operon Complementation –*all0396* and –*all0395* RSF1010 Plasmids (see sequences for further detail). These plasmids were used to create FC–*all0396* and FC–*all0395* complement mutants, respectively via introduction into the knock out, hence they lack the gene *all0396* and *all0395* repectively. *NptII*: *Neomycin Phosphotransferase II*. (Adapted from Malatinszky *et al.,* unpublished [15].)

SEQUENCES

Schizokinen Operon: Wild Type Genomic Sequence [8; 15]

455,014

3' Flank (antisense):
GTTAAAAAAGCGCGTCTAGTGAATTTGCTCATCTGCTTAACTCTCCTTGTGGAATACCTAGATGGCAAGGTAAACAAGTAGGATAGCCATTGATGGGGTG
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All0390 (antisense):
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TCCACATAACGTGAAAAATCAGATTTAAGAGAAACAGAG

All0391 (antisense):
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GAGGTGTTTGGCTGTGCTTTTGTTGAATATTTGCAGTTCTAAGGCGGCTTGGGTGACTGCTAGGAAGAAGCCGTAAACTATCCTCGCTAGGATGAGTAAG
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All0392 (antisense) (4 base overlap with *All0391*):
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All0393 (antisense) (7 base overlap with *All0392*):
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All0394 (antisense):
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ACCTCATCATCACATATTGTCTCACTCTTTTGACTAATCCCCGGCAAAATATTATCTAACAATTGATGACAAGAACGACGGTAATAATAACCTTGGTTGTCAC
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GCCACCCTGCGGGAACGGCAAAGCCGAACTGCGCGAAATAATCACCAT

All0396 (antisense) (1 base overlap with *All0395*):
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GCGCTGGAGTCACAATCAAA (Primer 176 antisense)
GGCGGCAAAAAGCGCACCACGCTACCAAATCTACCCCCTAATTCCACAATCAAACCCCGACGCAAACACTCAGCCTGAATACAACTAGCCAACTGCGGATG
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5’ Flank (antisense):
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5’ Flank (antisense, continued); *Alr0397*: AT GGATTGTGTCACTAGCCATA (Primer 124 antisense)
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ATTCC (end of 5’ Flank antisense)
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GCGGTGATTTGAACTTTAACGCCACACTGTTTAATGTAGGAGCAGTATACGCCCTCAATCCCCAACTGAGTGTATTTGCTAACTATGCTCAAGGTTTCTCAC
TATCAGATATTGGTTTAGCACTCCGCAACGCCCCACCAGGATTTTCTGTAGAATCTCTCAATCCCGAACCGCAAAAAGTAGATAACTATGAAATTGGCATTC
GCGGACAATGGGATACTGTACAAGCATCACTCTCAGCTTTTTATAACGAGTCCGACTTGGGTACAACTTTCACCGCGCCGGGGACTGTCATCCGCGCCCCA
GAGAGAATTTATGGTCTAGAAGCAGCCATTGACGCACAACCTAGTTCTACATGGCAAGTTGGCGGGACATTCACTCTCATTGGTGGTGAAATTGACAGCA
ATAATGATGGCGATTACGAATCATTAGATGGGTTTAGGATTCCCCCGTTGAAACTTACAGCCTATGTAGAAAATGAGACTTTACCCGGTTGGCGAAATCGT
CTACAAGCTTTGTACTCTGGTAATAGAGAAGTCTTTGGTAATAACAATACGGCCTTTGGTAGGAGACCTGTAGAAAGTTATTTAACAGTAGACTACATCAG
TAGTATTAAACTCGGTGCCGGAACATTACAACTGGGATTAGAAAACCTATTTAATAGTCAATATTTCCCCGTAGTTTCCCAATTGCAAGCAAATGATAGCGC
CTATGCTGCTGCTAGAGGAAGGACTTTGAGTATTAAGTATTCTTTTGATTGGTAA

469,228

Schizokinen Operon Knock-Out Plasmid (8744 bp) [15]

This plasmid was used to create the double-recombination schizokinen operon knock-out mutant, by replacing the genes *all0390* – *all0396* and *alr0397* with spectinomycin and streptomycin resistance marker genes. Hence it contains 5' and 3' flanking sequences for the operon, the same as in the wild type genomic sequence.

ACATGGCGATAGCTAGACTGGGCGGTTTTATGGACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTA
AACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGC

Neomycin Phosphotransferase II:
ATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGAT
GCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTCCAAGACGAGGCAGCGCGGC
TATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGC
AGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCG
ACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGC
CAGCCGAACTGTTCGCCAGGCTCAAGGCGCGGATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAA
ATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGG
CGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGA

GCGGGACTCTGGGGTTCGCTAGAGGATCGATCCTTTTTAACCCATCACATATACCTGCCGTTCACTATTATTTAGTGAAATGAGATATTATGATATTTTCTG
AATTGTGATTAAAAAGGCAACTTTATGCCCATGCAACAGAAACTATAAAAAATACAGAGAATGAAAAGAAACAGATAGATTTTTTAGTTCTTTAGGCCCGT
AGTCTGCAAATCCTTTTATGATTTTCTATCAAACAAAAGAGGAAAATAGACCAGTTGCAATCCAAACGAGAGTCTAATAGAATGAGGTCGAAAAGTAAATC
GCGCGGGTTTGTTACTGATAAAGCAGGCAAGACCTAAAATGTGTAAAGGGCAAAGTGTATACTTTGGCGTCACCCCTTACATATTTTAGGTCTTTTTTTATT
GTGCGTAACTAACTTGCCATCTTCAAACAGGAGGGCTGGAAGAAGCAGACCGCTAACACAGTACATAAAAAAGGAGACATGAACG

SacB:
ATGAACATCAAAAAGTTTGCAAAACAAGCAACAGTATTAACCTTTACTACCGCACTGCTGGCAGGAGGCGCAACTCAAGCGTTTGCGAAAGAAACGAACC
AAAAGCCATATAAGGAAACATACGGCATTTCCCATATTACACGCCATGATATGCTGCAAATCCCTGAACAGCAAAAAAATGAAAAATATCAAGTTTCTGAA
TTTGATTCGTCCACAATTAAAAATATCTCTTCTGCAAAAGGCCTGGACGTTTGGGACAGCTGGCCATTACAAAACGCTGACGGCACTGTCGCAAACTATCA
CGGCTACCACATCGTCTTTGCATTAGCCGGAGATCCTAAAAATGCGGATGACACATCGATTTACATGTTCTATCAAAAAGTCGGCGAAACTTCTATTGACA
GCTGGAAAAACGCTGGCCGCGTCTTTAAAGACAGCGACAAATTCGATGCAAATGATTCTATCCTAAAAGACCAAACACAAGAATGGTCAGGTTCAGCCAC
ATTTACATCTGACGGAAAAATCCGTTTATTCTACACTGATTTCTCCGGTAAACATTACGGCAAACAAACACTGACAACTGCACAAGTTAACGTATCAGCATC
AGACAGCTCTTTGAACATCAACGGTGTAGAGGATTATAAATCAATCTTTGACGGTGACGGAAAAACGTATCAAAATGTACAGCAGTTCATCGATGAAGGC
AACTACAGCTCAGGCGACAACCATACGCTGAGAGATCCTCACTACGTAGAAGATAAAGGCCACAAATACTTAGTATTTGAAGCAAACACTGGAACTGAAG
ATGGCTACCAAGGCGAAGAATCTTTATTTAACAAAGCATACTATGGCAAAAGCACATCATTCTTCCGTCAAGAAAGTCAAAAACTTCTGCAAAGCGATAAA
AAACGCACGGCTGAGTTAGCAAACGGCGCTCTCGGTATGATTGAGCTAAACGATGATTACACACTGAAAAAAGTGATGAAACCGCTGATTGCATCTAACA
CAGTAACAGATGAAATTGAACGCGCGAACGTCTTTAAAATGAACGGCAAATGGTACCTGTTCACTGACTCCCGCGGATCAAAAATGACGATTGACGGCAT
TACGTCTAACGATATTTACATGCTTGGTTATGTTTCTAATTCTTTAACTGGCCCATACAAGCCGCTGAACAAAACTGGCCTTGTGTTAAAAATGGATCTTGAT
CCTAACGATGTAACCTTTACTTACTCACACTTCGCTGTACCTCAAGCGAAAGGAAACAATGTCGTGATTACAAGCTATATGACAAACAGAGGATTCTACGC
AGACAAACAATCAACGTTTGCGCCGAGCTTCCTGCTGAACATCAAAGGCAAGAAAACATCTGTTGTCAAAGACAGCATCCTTGAACAAGGACAATTAACA
GTTAACAAATAA

AAACGCAAAAGAAAATGCCGATGGGTACCGAGCGAAATGACCGACCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATG
AAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCCTCGCGGACGTGCTCATAGTCCACGACGCCCGTGATTTTGTAGCCCTGGCCGACGGCCAGCAGGT
AGGCCGACAGGCTCATGCCGGCCGCCGCCGCCTTTTCCTCAATCGCTCTTCGTTCGTCTGGAAGGCAGTACACCTTGATAGGTGGGCTGCCCTTCCTGGTT
GGCTTGGTTTCATCAGCCATCCGCTTGCCCTCATCTGTTACGCCGGCGGTAGCCGGCCAGCCTCGCAGAGCAGGATTCCCGTTGAGCACCGCCAGGTGCG
AATAAGGGACAGTGAAGAAGGAACACCCGCTCGCGGGTGGGCCTACTTCACCTATCCTGCCCGGCTGACGCCGTTGGATACACCAAGGAAAGTCTACAC
GAACCCTTTGGCAAAATCCTGTATATCGTGCGAAAAAGGATGGATATACCGAAAAAATCGCTATAATGACCCCGAAGCAGGGTTATGCAGCGGAAAAGC
GCTGCTTCCCTGCTGTTTTGTGGAATATCTACCGACTGGAAACAGGCAAATGCAGGAAATTACTGAACTGAGGGGACAGGCGAGAGACGATGCCAAAGA
GCTCCTGAAAATCTCGATAACTCAAAAAATACGCCCGGTAGTGATCTTATTTCATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTT
CGCCAAAAGTTGGCCCAGGGCTTCCCGGTATCAACAGGGACACCAGGATTTATTTATTCTGCGAAGTGATCTTCCGTCACAGGTATTTATTCGGCGCAAAG
TGCGTCGGGTGATGCTGCCAACTTACTGATTTAGTGTATGATGGTGTTTTTGAGGTGCTCCAGTGGCTTCTGTTTCTATCAGCTCCTGAAAATCTCGATAAC
TCAAAAAATACGCCCGGTAGTGATCTTATTTCATTATGGTGAAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAGTTGGCCCAGGG
CTTCCCGGTATCAACAGGGACACCAGGATTTATTTATTCTGCGAAGTGATCTTCCGTCACAGGTATTTATTCGGCGCAAAGTGCGTCGGGTGATGCTGCCA
ACTTACTGATTTAGTGTATGATGGTGTTTTTGAGGTGCTCCAGTGGCTTCTGTTTCTATCAGGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAG
TTCTTCGCCCACCCCAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCC
GTAGAAAAGATCAAAGGATCTTC

Origin of Replication:
TTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTT
TCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTA
CATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCG
CAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAG
CGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCC
TGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCC

AGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTT
TGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCC
CCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCA
TTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACATGAT

5’ Flank; *Alr0397* (antisense, in part – gene disrupted):
GGAATCTTGAGCTACTTCAGTAGCCGTGAATGCCAAAATCAAACCTGTATCACTATCGAATAGCTCAAATTTGGGAAGGCTTTTCTCTCCCGTCACAGTCAC
CCGGATTGTATTGTCATTGTGATTAACCACAGTCACATTAGTTACACCTGTGGCTGGATTATTTTGGCTGAATGTATTTCCCTCAGACAAGTTGAGTTGAGC
GTTAGTGATATCAGCAATCAAACTATTGCCTTGATTTTGCGTTGATACCTGCAATTTTTCGGCGGCTGTACTATCTAATATCACCTCTAGACCTTGAACTGTA
GGAACAACTCTCACGCCAGTTACTTGTGTCAATTCCTGAGCGTTAGGTGCTGGTGATTGTGTATTTAGTTGTGTTGGGGATGGGGCTTCCTGAGTTTTGCC
AGGATGGCTGATTAATGACCAAACAGAACCTGTGAGCAAGAGAAGAAAAAGGATTTTTCCGGGTTTCATTTTCACTTCGCACCTAAAAGTTGCGACTGGA
T TATGGCTAGTGACACAATCC (Primer 124) AT

5’ Flank (continued): CGCCTATTATTATTGACTTGCATTAGCAATAGTTTTTG ATTTTATTAAAATATATCT (FurA Box)
ATTTCAGTACTTATACAAGTATTTTCTTGAAGAAGTATCTCAAAAAGGTATGAATTAACTACGAAAAAACCAATGATAAAAGCCGATAAAGCTCTTCCTACA
TAGTCTCTAGATATTTTGCTTTTTGTCTATTTACTGTTAGGAATTATTCTTGCTGACATTATCATTAATAACGCACACATACCTTTATAAGCGTGTTTTCGGTA
TCAGTTTCATTAGTATGATGATGCAACTAGCTTTAGATAAGAAGAATTAATGAATCAAAAATTTCTTATCTTTTTGTCAAGTTATTAGTAGACTTTTTCATTA
ATCTTCATGTAGGATGTTTAGCAGGTAGGAAATTAATGCAAGTAATTCTCAAATATTCTGTTCCCACTTACCATTAAGGCTCAACGCCTAGAGGATTTTCAC
A

TCATGGCTTCTTGTT

Spectinomycin Resistance:
ATGACATGTTTTTTTGGGGTACAGTCTATGCCTCGGGCATCCAAGCAGCAAGCGCGTTACGCCGTGGGTCGATGTTTGATGTTATGGAGCAGCAACGATG
TTACGCAGCAGGGCAGTCGCCCTAAAACAAAGTTAAACATC

Spectinomycin Resistance (continued); Streptomycin Resistance:
ATGAGGGAAGCGGTGATCGCCGAAGTATCGACTCAACTATCAGAGGTAGTTGGCGTCATCGAGCGCCATCTCGAACCGACGTTGCTGGCCGTACATTTGT
ACGGCTCCGCAGTGGATGGCGGCCTGAAGCCACACAGTGATATTGATTTGCTGGTTACGGTGACCGTAAGGCTTGATGAAACAACGCGGCGAGCTTTGA
TCAACGACCTTTTGGAAACTTCGGCTTCCCCTGGAGAGAGCGAGATTCTCCGCGCTGTAGAAGTCACCATTGTTGTGCACGACGACATCATTCCGTGGCGT
TATCCAGCTAAGCGCGAACTGCAATTTGGAGAATGGCAGCGCAATGACATTCTTGCAGGTATCTTCGAGCCAGCCACGTACGACATTGATCTGGCTATCTT
GCTGACAAAAGCAAGAGAACATAGCGTTGCCTTGGTAGGTCCAGCGGCGGAGGAACTCTTTGATCCGGTTCCTGAACAGGATCTATTTGAGGCGCTAAAT
GAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTGGCGATGAGCGAAATGTAGTGCTTACGTTGTCCCGCATTTGGTACAGCGCAGTAACCGGCA
AAATCGCGCCGAAGGATGTCGCTGCCGACTGGGCAATGGAGCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGAAGCTAGACAGGCTTATCTTGGACA
AGAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAATTTGTCCACTACGTGAAAGGCGAGATCACCAAGGTAGTCGGCAAATAA (end of
both resistance genes)

TGTCTAACAATTCGTTCAAGCCGACGGATATCGAGCTCGCTTGGACTCCTGTTGATAGATCCAGTAATGACCTCAGAACTCCATCTGGATTTGTTCAGAACG
CTCGGTTGCCGCCGGGCGTTTTTTATTGGTGAGAAT

3’ Flank:
TAATTCTCAATATTGAGGAAAACACTTGACAAAGATACTCATAATTAGGTTAGCTATATAGTTGTTATAGGCAACAGAAAGCAATCTTCAAGGGCAGTAAT
AGTTAACTATTACTGCCATTAACTATCAGGAGGTCAGGAATTGCCAGCGATGGACTACGCCCCACCTACGGTGATCGCATCTCAAAATCTGCAAATTGCCT
ATGATGGCAAAATTATCGTTCCTGACCTCTCAGTCGCGTTTTCTCTGGGAGAAATCACCGCCTTACTAGGGCCGAATGGTTCTGGCAAAAGTACCGTTCTCC
GCACCCTTGCCAGACTTATGCAACCAAGCAAGGGTACTATCTATCTGCATGGGCGTGATATTAGCCACATACCCACAAAAGAGTTAGCCAAACAACTGACG
ATATTACCCCAATCTTCAGAAGCACCTCCAGGTGTAACTGTGT GGGAATTGATTGGTTATGGAC (Primer 125 antisense)
GCTATCCCCATCAGAATTTATTAGGGGGTTTTTCTCAAAAAGATATCGCTGCGATGGAATGGGCGTTGGCTGTGACTGGCTTAGAACCTCTAGCAAACAGA
ATAGTCGATACCCTCTCCGGTGGGGAACGCCAAAGGGCTTGGATTGCAATGGCCTTAGCACAGCAAACCCAAGTGTTATTACTCGATGAACCCACAACGT
TTTTAGATATCCGCCATCAAATAGAAGTTTTAGCCTTAGTACGACGACTCAACCAAGAACACGGAATTACCGTCGGTTGGGTGTTACATGACTTGAATCAA
GCCGCAGCCTATAGCGATCGCCTGATCATGCTCCAACAAGGAAAAATAGTCACCGTAGGTACTCCCCAAGAAGTAATGACAGCCAGTAATATTCAGCAGG
TGTTCGGCGTAGAAATGACCGTGATTCCCCACCCCATCAATGGCTATCCTACTTGTTTACCTTGCCATCTAGGTATTCCACAAGGAGAGTTAAGCAGATGA
GCAAATTCACTAGACGCGCTTTTTTAAC G

LacZ:
CACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGC
GAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGATAA

GCTAGCTTCACGCTGCCGCAAGCACTCAGGGCGCAAGGGCTGCTAAAGGAAGCGGAACACGTAGAAAGCCAGTCCGCAGAAACGGTGCTGACCCCGGA
TGAATGTCAGCTACTGGGCTATCTGGACAAGGGAAAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTT

Schizokinen Operon Complementation RSF1010 Plasmid (17,564 bp) [15]

This was used to create the full complement mutant, via introduction into the knock out; hence it contains genes *all0390* – *all0396*.

CGCCTATTATTATTGACTTGCATTAGCAATAGTTTTTGATTTTATTAAAATATATCTATTTCAGTACTTATACAAGTATTTTCTTGAAGAAGTATCTCAAAAAG
GTATGAATTAACTACGAAAAAACCAATGATAAAAGCCGATAAAGCTCTTCCTACATAGTCTCTAGATATTTTGCTTTTTGTCTATTTACTGTTAGGAATTATT
CTTGCTGACATTATCATTAATAACGCACACATACCTTTATAAGCGTGTTTTCGGTATCAGTTTCATTAGTATGATGATGCAACTAGCTTTAGATAAGAAGAA
TTAATGAATCAAAAATTTCTTATCTTTTTGTCAAGTTATTAGTAGACTTTTTCATTAATCTTCATGTAGGATGTTTAGCAGGTAGGAAATTAATGCAAGTAAT
TCTCAAATATTCTGTTCCCACTTACCATTAAGGCTCAACGCCTAGAGGATTTTCACA

All0396:
ATGCAACTCAATCACAATGGCGTACACTCTGAAGGCCACTCTTTCGTGCTGCCGCAGATATGTCCATCGCAGAGTAGTGTAGAGTCTGATTTTCCCAGTAA
TCGCGTGCGTTCTGTACCTGGTGCGGCATCAAGCCACTATTTAGAGCGTCAGCAAGCACGAGAGTCCAACGCTAGAAGCTATCCCCGGCGGATTCGGATT
GCCATTAGTGAGGCTCAGGGTATTTATCTCAAAGATGCTGATGGCAATGTATATATTGATTGTTTGGCTGGTGCAGGTACTTTAGCATTAGGACACAATCA
CCCAGTAGCAATAGAAGCGATGCGCCGGGTGCTAGATACGGGTTTACCTTTGCATACTCTAGATTTGACTACACCAGTTAAAGATCAGTTTGTCGAAGAAA
TTTTTGCTAGTTTACCTGCGGAATTTGCTCAGAATGCCAAGATTCAGTTTTGTGGGCCTTCGGGAGCAGATGCTGTAGAGGCGGCGATTAAACTGGTGAAA
ACTGCTACTGGCCACCGCAGTGTGTTGTCATTTCATGGTGGTTATCATGGGATGACTCATGGAGCATTGAGTTTAACAGGCAATCTCAATCCTAAACAAGC
GGTGACAGGGTTAATGCCAGATGTCCACTTTTTGCCTTATCCCTATCATTATCGTTGTCCCTTTGGTTTGGGTGGAGAAGCTGGACAAATTACCAGCAGCCG
TTATATAGAATCGATTTTGGATGATCCTGAAAGTGGGATTGTCACACCGGCGGCGATGATTTTGGAAGTTGTGCAAGGGGAAGGTGGGGTGATTCCTGCA
CCTGATGATTGGTTGCGAGAGATGCGCCGGATTACCCGCGATCGCCATATTCCCCTCATTGTTGATGAGATTCAAACAGGCTTAGGACGGACTGGTAAACT
CTACGCTTTTGAGCATTCAGGGATTGTGCCAGATGTGTTGTTACTCTCCAAAGCTATTGGTGGGAGTTTACCTTTATCGGTGGTTTTGTACAACAAGGCACT
AGATAAATGGAGTCCAGGCGCACACGCAGGGACATTCCGGGGGTAATCAAATGGCAATGGCAGCCGGAACAGCGACACTACAGTATATTTTAGAAAATTC
CTTGACTGAACACGCGGCAGCGATGGGCGATCGCTTGTTGAAACATCTGCACCAAATTCAAGGGGAAACTTACTGTATTGGAGAAGTGCGGGGACGCGG
TTTGATGGTTGGTGTGGAGATTATTAATCCCCAAGCATCGGCTGATCGGCGTGGGAAGTATCCTGTACATCCGCAGTTGGCTAGTTGTATTCAGGCTGAGT
GTTTGCGTCGGGGTTTGATTGTGGAATTAGGGGGTAGATTTGGTAGCGTGGTGCGCTTTTTGCCGCC TTTGATTGTGACTCCAGCGC (Primer 176)
AGATTGATAGTATTAGTGAGATTTTTTCGTCGGCGGTACAGGCGGCGGAGAAGCAGGTTTTATCGGTTCTTTCGCAGGTTTCTTGA

All0395 (1 base overlap with *All0396*):
ATGGTGATTATTTCGCGCAGTTCGGCTTTGCCGTTCCCGCAGGGTGGCGCAAAGGAGGACACTTGCGTGGGCGGGTCTCCCGACTTGAGCAAAGTGTCCG
TCACGCAAAGAAAGAAGTTTGATGAGTTTTTTGTTGCAGGAGAGGGTTTGCGGGAGGCGATCGCTGCTGCTGGAGAGGTGTTGATTGATTATTTTGCTAC
TCAAGAAAAACCCTATAGTGGTAGGAGTCCCCAGGATTTAACTAGGACTATT GCAGATATCGCTGTGTGTCC (Primer 175 antisense)
TGATGAGGGGGTGGCTTTGAGTCAGGTTTTGGCTGAGGTGGGGGAGAATATCATCAAGCATTCGGTGGTGGTAACTCATCCTACTTGTATGGCTCATTTG
CATTGTCCACCGTTATTACCTGCTGTGGCGGCTGAGGTGTTGATTAGTGGGACTAATCAATCTCTGGATTCTTGGGATCAAAGTCCGGCTGCTACTGTTTTG
GAACAGCAGGTGGTAAATTGGCTGTGTGCAAGTTTTGGTTATGATGCTGATGCTGATGGTATATTTACCAGTGGCGGTACGCAATCGAATTTTATGGGGT
TGCTCCTAGCGCGTGATGCTTACGCACGTCATCAGTTAAACTGGTCTGTGCAGCAGCAAGGATTACCACCAGAAGCTCAACGTTTTCGGATTCTCTGTTCTC
AAGCTGCTCATTTCACCATTAGTCAAGCTGCTTCTTTACTCGGTTTGGGACAGCAAGCTGTGGTAACGGTGGAGACTGATAGTGATTATCAGCTTTGTGCG
GCGGCGGTAGAACAGAAGTTAGAAGAATTGCAGCAACAGAATTTATTACCCATTGCTTTAGTTGCAACTGCGGGAACTACGGATTTTGCTAGTATTGACA
AATTACCAGAATTAGCCGCCTGTGCTGAGAAATATGGCCTGTGGTTTCATGTGGATGCGGCTTTTGGTGGTGCATTGGTGATGAGCGATCGCCATCGAGA
TAAACTAGATGGTATTGCTTTGGCTGACTCGATTACAGTCGATTTCCATAAACTGTTTTACCAGCCAATTAGCTGTGGTGCTTTTTTAGTTAAACAGCGTCAA
AACTTTGATTTAATAAAGCTACACGCTGATTATCTCAACCCTGAAACCAACGAAGTAGCCAGCATTCCCGACTTAGTAACTAAGTCCATACAAACCACCAAG
CGATTCGACGCGCTCAAATTGTTTGTTTCTCTCCGCACTCTGGGGAGGAAACAATTTGCACAGATGATTGATACAACAATTGAACTAGCCAAGGAAACCGC
TAGTTTAATTGACGCTGAACCTGCATTGGAGTTGGCAAACAATCCTACCATTAACGCTGTTGTTTTTCGCTATCTTCCTAGTGAAACACCAGCACACATAGA
TAGTACAACTTGGGCAAATCAAATCAATAGCCATATTCGCATGAGTTTACTGCAACAAGGTATCGCAGTCATCGCACAGACCAAAATTGGTCAACTTACCT
ATCTAAAGTTTACCTTGCTGAATCCTCAGACGGCGATCGCTGATATTCAAGAGGTTCTCAACTCTATCACAACGATAGGTGAGAAGTATTTATTTCACGCAC
AAGAGAGTAAAGAAGGGTTAAGCCTCTAA

GCTCGACACTCTCATTCTCTCCGCGTCTCTGCGTGAGCAAAAAATAGGAGAGAGGGAGAGCGAACAACTCTCCTCACTCCCAACATTAAATAACTTTCACC
CAAACAATACACTAGAC

All0394:
ATGACACCATACACAATTGCCAAACCAACCTCGGTGCTGGTGGAATATCGCACTGATAAACAAATTGCAGAACAAGCAACTATTCACAGCTTTTTGAATTG
CTATCTGCGCGAAACCAACACGGGTAAACTCATCACCACCGCGACAAAAGACGCTGATATTTTAGAAGTTTTCCAAAACACAAATACAAAATCGCTCATCT
GCTGTGAGTTAAAGCAACAAAATCTGAGATTATTAATCGGTTTAAGATATTACTCACCCACAGGTAGACATTTATTTGCTTTTCCCCTTTATTATCAAGTAGA
TAAAGGTAATTTACTTGAACTGGATTATCTCACCTTAGCAACGCTCATTACTAAAGAATTATCCTTAGCTGGTGGTAGCAATAGCCATCAAGATGAACTGAT
TTTGCGAGTGATTCAAAGCTGCAATCACATTGAATATTTCGTTCAGAAACGGCGACAAGATATAGAAAAGCTCTATACTTTTAACAGTAATTTCATCGCATC
TGAACAAGCTTTAGTTTTTGGACACCATTTACACCCCACCCCAAAAAGTCGGCAAGGTTTTGCTGACCATGAATTGTCAATTTATTCGCCAGAATTAAAAGG
TAGTTTTCCTCTGCATTACTTCCGCATTCATCAGTCAATGGTACTGGAAGGTTCGCAGCTATCACAGACAGCAACAACGCTGATTAAATCAGAATTATTGGC
TGATCCAAAGGTTGATAATCAATTCAAAAATACTTACTGTAATGAAGATGAATATGCTTTATTACCGATACACCCTTGGCAAGCTAATTATTTACTGCAACA
GCCACAAATTCAACAATTAATTAAGCAAGAAATATTGCAAGATTTAGGTTTAGTCGGTCGAGCATATCAACCTACATCTTCAATTCGCACTGTCTATCATCC
AGATGCGGCATTTATGTTAAAATTGTCGCTGAATATCAAAATTACCAACTCTGTCCGCACTAATTTATATAAAGAATTAGAACGGAGTTTAGAAGTTCATCA
AATTTTAACAAGTGAAATTGGGCAACAACTTTATCAGCGTTTTCCTGAGTTCCAAATCATCACTGATCCTGCTTATATCACCTTAAAAATTGATGGTGTTGCT
GTTGATGGTTTCTCAACAATTCTGCGAGAAAATCCGTTTTTAAATAATCCCCAGACGGATGCAACTTGTGTAGTAGCTTTATGTCAAGACTCTATTTTGGGT
AACGGTTCACGATTAGCACGGATTATTGAAGAACTAGCACAACAAGAAAACCGTTCGACTGAGGCGGTGAGTTTGGATTGGTTCAACCGTTATTTACAGA
TTTACTTAGAGCCAATTCTCTGGTTATATTTCACTTACGGGATAGGACTAGAAGCCCATCAGCAAAATAGTGTTGTACAGTTAAAAAATGGCTATCCTGAG
AAGTTCTTTTATCGTGACAACCAAGGTTATTATTACCGTCGTTCTTGTCATCAATTGTTAGATAATATTTTGCCGGGGATTAGTCAAAAGAGTGAGACAATA

TGTGATGATGAGGTTATTGATGAACGATTGACTTACTACTTGTTTTTTAATAATTTGTTCGGGTTAATTAATGCTTTTGGTGTAGCTGGACTTGTAGATGAG
GAATTACTCTTAGG GGAGTTGCGAAACATCTTAGGC (Primer 62 antisense)
AAATATTCTGAACATTCTTTAGTGAATAATTTACTTTTTCAATCACAGTTACTTTGTAAGGCTAATCTTCTCACCCGGTTTCATAATCTGGATGAACTGGTGG
GGCCAGTTTCTACACAGTCTGTCTATGTTGCTGTTGATAATCCTTTGATGTAA

ACGCAGAGGGACGCAGGGGTTTACGCAAAGTAACGCAGAGAGTTTATGAGAGGAATTTGAGGCTGAAGTTT

All0393:
ATGTCGGTTAGTATGATTAATTACAGTTACGCAAGGTTTGATGCTGCAATCAATAAAACTATTGCTTTTCGTCCGGTGGTTTTGGAGGAGGATTTAAACCTG
ATTCATAACTGGATGAATCAACCTCATGTAATTCCTTTTTGGAATTTAGCCTTTGATTTGGAACGGATGCGGGAGCATTTACAAAGGACTTTAGCAGATAAA
CATCAAACTCTTTATATTGGTTGTTTAGATGATGAACCGATGAGTTATTGGGAGTCATACTGGACAATTGATGATATTGTGGCTCGACATTATTCAGCAGAA
GCGACAGATCAAGGTATTCATTTGTTAATTGGGGAAACCAAATTTTTAGGTAAAGGTTATGCTTTGCCACTGTTACGGGCAATGGTATTTTTTCAATTTGAA
AATACAGCAACTCAAAAGATTATTGCTGAACCAGATATTCGCAATCAAAAGATGATTCATGTTTTTGAAAAGTGTGGTTTTGAGTTTCAGAAAGAGATTGA
ATTACCGGATAAATTTGGGGCGTTGATGTTTTGCGATCGCCAGCTATTTTTTCAGAGGTGGAAAGCATGGTAA

All0392 (7 base overlap with *All0393*):
ATGGTAAATTGTGTTTATGACTTGATTGGTATTGGTCTTGGGCCTTTTAATTTAGGTTTAGCGGCGCTGTTAGAACCAATAACAGAGATTAAGTCTTTATTC
CTCGAACAAAAGCCTCAATTTCAATGGCATCCAGGGTTGTTACTTGAGGGGACGACAATTCAAGTACCATTTTTGGCAGACTTGGTGACAATGGCTGAACC
TAGTAGTAAATTCAGCTTTCTTAGTTACCTGAAAGCTAAGTCTCGTCTCTATAATTTTTACTTCTGGGAAGAGTTTCATATTCCCAGGCGAGAATATAACGAT
TACTGTCAATGGGTAGCAAAGCAATTACCAAATTGTTACTTTGGTGAACAGGTAAAAAGTATTGATTGGGATGAAAAAGCGCAGGAATTTATAGTGTCTG
GTACTAATTTCATCTACCGTTGTCGCAATTTGGTTTTAGGCGTTGGGACTGTTCCCTACATACCGCCTTGTTTCCGTGATTTAGTATCAGAAAATGTTTTTCA
CTCGTCCAAATTTCTCCATCAAAAAGTAAGCTGTCGTCAAGCGAAATCAATCACAGTTATCGGTTCTGGACAAAGTGCAGCCGAGGTTTTTTATGAACTATT
GCAAGAGCAAGAAAACTATGATTATCACCTAGAATGGCATACTCGTTCTTCCGGCTTTTTCCCAATGGAATATTCCAAATTGGGGTTAGAACATTTTTCACC
CGATTACATCCATTATTTCTACCATCTGCAACCAGAACAGCGAGACGAACTATTAACTAAACAAGGGTTGCTGTATAAGGGAATTAGCTTTAACACCATAG
CCAAAATATATGATTTGCTTTACGAGCGTTCCGTTGCTGATAATTACCCTGATGTTAAATTACTCTCTGGAGTAGAAGTTAAAGACATAGAACCCACTGCTG
AAGGTTATCGTCTCACCTATCGCCATTCTCATCAGCATCAACCATTTATTCATGAAAGCGATCGCATTATCTTAGCTACAGGCTATCATCACGCCACCCCTAA
TTTTATGGCAGATATCCGCGATTTGCTGCAATGGGATGAAAAAGGGCGTTACAAGGTAAATTTTGACTATCATCTCTCCCTGACTCAAGACATCCCCAACC
GGATTTTTGTCCAAAACGCTGAGTTACATACCCACGGGATTGGTGCGCCAGATTTGGGTTTAGGTTGTTACCGCAATTCTGTCATTATCAACTCCTTAACGG
GACGCAATACGTACCCAGTACAACAGCGCAACGTCTTTCAGCAATTTGGTCTAGTCCCATGA

All0391 (4 base overlap with *All0392*):
ATGAAGCATCGGCTACCCCTCATCTCAGGTGCATTATTTTTATGTGTGTTCTTGAGTATTTTCAACGAGGTTTTACTGTCACCCTTTTATCCCCAGTTTTCCG
TAAAGTCTTTGGCGTGACAGATTTAGCCTACACCGGTTACTACATCTTTGTGTGTCGGTTAACCGTGGTGCTGTGTGCGCCTGTGTGGGGAGTGTTGTCAC
GCCGCTTTGAAGTCAAACACCTACTCTTTGTTGGACAATTGGGTGCAGCCTTCATGACAGCCTTGATGGGTACAAGCAGCAGTGTAGAGCAATTTCTGATG
TACACAATTTTGCTGTTACTGTGCAAAAGCAGTTATTTGCTAGTGTATCCCCTCATTATCCAACTAGGAGGTGAAGAAAAACGAGCTGCGATCGCCGGCAC
ATACCAAGCTGTATTTCATGGTGCAATTATCATCGCTACCATCGTCGGCGCATTTATGGTCAATATTGACACACCCTTAATTATTTTTTACGGGATTGCCGCA
GCCGATCTTTTACAACTTGCCATCTGTGCTTATATGTTGCGGGGGTGTATCTACCGCAGGGGGGCAGGGAGCAGGGGGCAGGGGAGAAAATCAGCCAGTA
GCACCAAACCAACTGGGTTACATCATCGCTATCGGGGTAGTAATTCTCACCTTCCAACTAGCCAATAACTTAGTCCGTCCCTACTTCACAGCCTATGTTACC
GCCGAACCACTAAAAGTTGACCTGCTCACAAGTAGCTTGTTATTTCTGATACCCAGTGTAATGGCGATCGCTGCCTTACCCTATATTCGTCAAGCCTGTCGT
CCCGAACGCCTCCATACCATCTACTTAGGAAGTTTAAGCCTACTCATCGTCAGCTTAGGCATACAAGGATTATCATCCAACCTACCCTTACTCATCCTAGCGA
GGATAGTTTACGGCTTCTTCCTAGCAGTCACCCAAGCCGCCTTAGAACTGCAAATATTCAACAAAAGCACAGCCAAACACCTCCACTTCAACTACAGCCTTG
CCACCTCCTTCGCCAACATCGGACACTTAGGCGCACCCCTTTTAGCCTCCTGGCTAGTCAACACCCACAGCCTAGCCTCCCCATTCATCGCCGCCACCATAAT
TTGCTGTCTAAACCTCCTATTCTTTCGCTATGTTCCGAAGGCAGGCGGCAGGCGGCAGGCGGCAGAAGGATTTTAA

CTCTGTTTCTCTTAAATCTGATTTTTCACGTTATGTGGAAAAATCTACGCTTAACCTAACCCCCTAACCCCCTTCCCTAGTAGGGAAGGGGGAAAATTCAAA
GCCTCTCTCCGTGTCGGGGAGAGGTTTGGAGAGGGGTTATCCAGAACCCGTGAAAATTCCCAAACAAAAATATGCAAAACCTAACCAAAATCCTCCAATA
CCCACGCTGGCAAACAGTTAGCCAAAAACTCCTAGCCAAA

All0390:
ATGCTCTCCGAATTCATGTATGAAGAAATCATCAAACCGGAGACAATAGAACAAACAGCAGAATATACCCTCTACCACCTCGCCCTCCCCGAAGGCATCGC
CTACAACTTCCAAGCCAAAAAACGCCTATTCGACAGCTACCGCGTCATCCCCGCATCTATCCAACGACGAGAAGCGGGAGAATTTTCCCCAGCATTCAACC
CCCTGCAATTCGTCCTCGATATTCACACATTTGTAGGAATGACGGCTGAAACCACAGCTCATTTAATCAAAGAACTCAGCAACACCCTACTAGCCGACGCAC
ATATTCAAACCAAAAAAGAAACCCAAGATATAGACTTACTCAATTTAGATTACCCCTCATTAGAAGGGGAAATGGAAGGACACCCCTGGATAACCTTCAAT
AAAGGACGCATTGGTTTTGGCTATGACGATTACCTAGCCCACGCACCCGAAAGTAAACAGCCAGTTTCCCTCTTTTGGATTGCTGTTAGCAGTGAACGCGC
CCAATTTAACGCCATCCCAGGACTAGATTATGTCACCCTCATTCAAGAAGAATTAGGCGCAGAAAGTCTTGCGGAATTTACGGCTATTTTAGAACAACGTC
ATTTCAGCCCCGCAGATTATTATTTTCTTCCCGTCCATGATTGGCAATGGAAAAATATCATCACCCTTTTGTTTGTGGAAGAAATAGCCACAGGTGGAATTA
TTCCCCTCGGCTACAGCCAAGATAAATATTTACCCCAACAATCGATTCGCACCTTTGCTAATATCAGCTATCCGCAGAAACGGTACGTTAAATTACCCCTGA
GCATTTTAAATACTCTCGTTTACCGTGGTTTACCAGGCGATCGCACACAGGTTGCCCCACTGGTTACAGAATATGTAAAATCGATTTGTGATCATGACCCTT
TCCTCAAAGATGAGTGTCGCTTAATTCTTCCTGGGGAAATTGCCAGCATCAACTACGATCATCCATACTACAGCCAGCTTTCAGGCGCACCTTACCAATATA
AGGAAATGTTGGGTTGTCTATGGCGCGAGAGTGTTTTAGCTTACACCAAAGCCGATGAACGTCCAATTACTTTAGCGTCTTTATTACATATCGATGGTAAC
GGTCAACCCTTTATTTCTCAACTGGTAGAACGTTCTGGACTCAGTTTAGATGAATGGTTATCTCGACTATTCAACACGATTTTACCGCCATTACTACATTACC
TCTACCGCTACGGCGTGGTTTTCTCCCCCCACGGTGAAAACACAATTTTGGTGCTGAAGGATTTTGCCCCCCATCGGTTAGCGATGAAAGATTTTGTCGATG
ATGTAAATATCAGTCGTCATCCCCTACCGGAATTAGAGACTTTAACACCACAACTCAAAGCCGTTTTGTTGACTGAACCACCAGAAGGATTATGTCAATTTA
TCTTTGCTGGCTTGTTTATCTGTCACCATCGTTATCTGTCTGATTTATTGGCAGACTACCACAACTACCCAGAACAGACCTTCTGGACAAAAGTCAGAGAGA
CGATTTTAAGCTATCAAAGTCGTTTCCCCGAAATGCAAGACAGATTTGAGTTATTCAACTTGCTAGCACCCCAATTTACCAAGCTGTGCTTGAATCGCAATC
GCCTGATTACTTACGGTTACGCCGATGATGGCGATCGCCCCCATGCGGCCGCCTTCGGTAAAGTGAATAATGCTTTGTATACAGTAGCCCAATTAATGCCA
ATGGAGCGCTAA

TAATAATTCGCTCGGTTGCCGCCGGGCGTTTTTTATTACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAA
GTAAACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGC

Neomycin Phosphotransferase II:
ATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGAT
GCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTCCAAGACGAGGCAGCGCGGC
TATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGC
AGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCG
ACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGC
CAGCCGAACTGTTCGCCAGGCTCAAGGCGCGGATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAA
ATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGG
CGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGA

GCGGGACTCTGGGGTTCGCTAGAGGATCGATCCTTTTTAACCCATCACATATACCTGCCGTTCACTATTATTTAGTGAAATGAGATATTATGATATTTTCTG
AATTGTGATTAAAAAGGCAACTTTATGCCCATGCAACAGAAACTATAA

RSF1010 Backbone:
CTGAAAGCGACCAGGTGCTCGGCGTGGCAAGACTCGCAGCGAACCCGTAGAAAGCCATGCTCCAGCCGCCCGCATTGGAGAAATTCTTCAAATTCCCGTT
GCACATAGCCCGGCAATTCCTTTCCCTGCTCTGCCATAAGCGCAGCGAATGCCGGGTAATACTCGTCAACGATCTGATAGAGAAGGGTTTGCTCGGGTCG
GTGGCTCTGGTAACGACCAGTATCCCGATCCCGGCTGGCCGTCCTGGCCGCCACATGAGGCATGTTCCGCGTCCTTGCAATACTGTGTTTACATACAGTCT
ATCGCTTAGCGGAAAGTTCTTTTACCCTCAGCCGAAATGCCTGCCGTTGCTAGACATTGCCAGCCAGTGCCCGTCACTCCCGTACTAACTGTCA

RSF1010 Backbone (continued); RSF1010 OriV Origin of Replication:
CGAACCCCTGCAATAACTGTCACGCCCCCCTGCAATAACTGTCACGAACCCCTGCAATAACTGTCACGCCCCCAAACCTGCAAACCCAGCAGGGGCGGGG
GCTGGCGGGGTGTTGGAAAAATCCATCCATGATTATCTAAGAATAATCCACTAGGCGCGGTTATCAGCGCCCTTGTGGGGCGCTGCTGCCCTTGCCCAAT
ATGCCCGGCCAGAGGCCGGATAGCTGGTCTATTCGCTGCGCTAGGCTACACACCGCCCCACCGCTGCGCGGCAGGGGGAAAGGCGGGCAAAGCCCGCT
AAACCCCACACCAAACCCCGCAGAAATACGCTGGAGCGCTTTTAGCCGCTTTAGCGGCCTTTCCCCCTACCCGAAGGGTGGGGGCGCGTGTGCAGCC

RSF1010 Backbone (continued): CCGCAGGGCCTGTCTCGGTCGATCAT

RSF1010 Backbone (continued); *Mobilisation Protein C* (antisense):
TCAGCCCGGCTCATCCTTCTGGCGTGGCGGCAGACCGAACAAGGCGCGGTCGTGGTCGCGTTCAAGGTACGCATCCATTGCCGCCATGAGCCGATCCTCC
GGCCACTCGCTGCTGTTCACCTTGGCCAAAATCATGGCCCCCACCAGCACCTTGCGCCTTGTTTCGTTCTTGCGCTCTTGCTGCTGTTCCCTTGCCCGCACCC
GCTGAATTTCGGCATTGATTCGCGCTCGTTGTTCTTCGAGCTTGGCCAGCCGATCCGCCGCCTTGTTGCTCCCCTTAACCAT

RSF1010 Backbone (continued):
CTTGACACCCCATTGTTAATGTGCTGTCTCGTAGGCTATCATGGAGGCACAGCGGCGGCAATCCCGACCCTACTTTGTAGGGGAGGGCGCACTTACCGGTT
TCTCTTCGAGAAACTGGCCTAACGGCCACCCTTCGGGCGGTGCGCTCTCCGAGGGCCATTGCATGGAGCCGAAAAGCAAAAGCAACAGCGAGGCAGC

RSF1010 Backbone (continued); *Mobilisation Protein A*:
ATGGCGATTTATCACCTTACGGCGAAAACCGGCAGCAGGTCGGGCGGCCAATCGGCCAGGGCCAAGGCCGACTACATCCAGCGCGAAGGCAAGTATGCC
CGCGACATGGATGAAGTCTTGCACGCCGAATCCGGGCACATGCCGGAGTTCGTCGAGCGGCCCGCCGACTACTGGGATGCTGCCGACCTGTATGAACGC
GCCAATGGGCGGCTGTTCAAGGAGGTCGAATTTGCCCTGCCGGTCGAGCTGACCCTCGACCAGCAGAAGGCGCTGGCGTCCGAGTTCGCCCAGCACCTG
ACCGGTGCCGAGCGCCTGCCGTATACGCTGGCCATCCATGCCGGTGGCGGCGAGAACCCGCACTGCCACCTGATGATCTCCGAGCGGATCAATGACGGC
ATCGAGCGGCCCGCCGCTCAGTGGTTCAAGCGGTACAACGGCAAGACCCCGGAGAAGGGCGGGGCACAGAAGACCGAAGCGCTCAAGCCCAAGGCATG
GCTTGAGCAGACCCGCGAGGCATGGGCCGACCATGCCAACCGGGCATTAGAGCGGGCTGGCCACGACGCCCGCATTGACCACAGAACACTTGAGGCGC
AGGGCATCGAGCGCCTGCCCGGTGTTCACCTGGGGCCGAACGTGGTGGAGATGGAAGGCCGGGGCATCCGCACCGACCGGGCAGACGTGGCCCTGAAC
ATCGACACCGCCAACGCCCAGATCATCGACTTACAGGAATACCGGGAGGCAATAGACC

RSF1010 Backbone (continued); *Mobilisation Protein A* (continued); *Mobilisation Protein B*:
ATGAACGCAATCGACAGAGTGAAGAAATCCAGAGGCATCAACGAGTTAGCGGAGCAGATCGAACCGCTGGCCCAGAGCATGGCGACACTGGCCGACGA
AGCCCGGCAGGTCATGAGCCAGACCCAGCAGGCCAGCGAGGCGCAGGCGGCGGAGTGGCTGAAAGCCCAGCGCCAGACAGGGGCGGCATGGGTGGA
GCTGGCCAAAGAGTTGCGGGAGGTAGCCGCCGAGGTGAGCAGCGCCGCGCAGAGCGCCCGGAGCGCGTCGCGGGGGTGGCACTGGAAGCTATGGCTA
ACCGTGATGCTGGCTTCCATGATGCCTACGGTGGTGCTGCTGATCGCATCGTTGCTCTTGCTCGACCTGACGCCACTGACAACCGAGGACGGCTCGATCTG
GCTGCGCTTGGTGGCCCGATGA

RSF1010 Backbone (continued); *Mobilisation Protein A* (continued); *Replication Protein B* (3 base overlap with *Mobilisation Protein B*):
ATGAAGAACGACAGGACTTTGCAGGCCATAGGCCGACAGCTCAAGGCCATGGGCTGTGAGCGCTTCGATATCGGCGTCAGGGACGCCACCACCGGCCAG
ATGATGAACCGGGAATGGTCAGCCGCCGAAGTGCTCCAGAACACGCCATGGCTCAAGCGGATGAATGCCCAGGGCAATGACGTGTATATCAGGCCCGCC
GAGCAGGAGCGGCATGGTCTGGTGCTGGTGGACGACCTCAGCGAGTTTGACCTGGATGACATGAAAGCCGAGGGCCGGGAGCCTGCCCTGGTAGTGGA
AACCAGCCCGAAGAACTATCAGGCATGGGTCAAGGTGGCCGACGCCGCAGGCGGTGAACTTCGGGGGCAGATTGCCCGGACGCTGGCCAGCGAGTACG
ACGCCGACCCGGCCAGCGCCGACAGCCGCCACTATGGCCGCTTGGCGGGCTTCACCAACCGCAAGGACAAGCACACCACCCGCGCCGGTTATCAGCCGT
GGGTGCTGCTGCGTGAATCCAAGGGCAAGACCGCCACCGCTGGCCCGGCGCTGGTGCAGCAGGCTGGCCAGCAGATCGAGCAGGCCCAGCGGCAGCAG
GAGAAGGCCCGCAGGCTGGCCAGCCTCGAACTGCCCGAGCGGCAGCTTAGCCGCCACCGGCGCACGGCGCTGGACGAGTACCGCAGCGAGATGGCCGG
GCTGGTCAAGCGCTTCGGTGATGACCTCAGCAAGTGCGACTTTATCGCCGCGCAGAAGCTGGCCAGCCGGGGCCGCAGTGCCGAGGAAATCGGCAAGG
CCATGGCCGAGGCCAGCCCAGCGCTGGCAGAGCGCAAGCCCGGCCACGAAGCGGATTACATCGAGCGCACCGTCAGCAAGGTCATGGGTCTGCCCAGC
GTCCAGCTTGCGCGGGCCGAGCTGGCACGGGCACCGGCACCCCGCCAGCGAGGCATGGACAGGGGCGGGCCAGATTTCAGCATGTAG

RSF1010 Backbone (continued):
TGCTTGCGTTGGTACTCACGCCTGTTATACTATGAGTACTCACGCACAGAAGGGGGTTTTATGGAATACGAAAAAAGCGCTTCAGGGTCGGTCTACCTGAT
CAAAAGTGACAAGGGCTATTGGTTGCCCGGTGGCTTTGGTTATACGTCAAACAAGGCCGAGGCTGGCCGCTTTTCAGTCGCTGATATGGCCAGCCTTAAC
CTTGACGGCTGCACCTTGTCCTTGTTCCGCGAAGACAAGCCTTTCGGCCCCGGCAAGTTTCTCGGTGACTGATATGAAAGACCAAAAGGACAAGCAGACC
GGCGACCTGCTGGCCAGCCCTGACGCTGTACGCCAAGCGCGATATGCCGAGCGCATGAAGGCCAAAGGGATGCGTCAGCGCAAGTTCTGGCTGACCGAC
GACGAATACGAGGCGCTGCGCGAGTGCCTGGAAGAACTCAGAGCGGCGCAGGGCGGGGGTAGTGACCCCGCCAGCGCCTAACCACCAACTGCCTGCAA
AGGAGGCAATCA

RSF1010 Backbone (continued); *Replication Protein A*:
ATGGCTACCCATAAGCCTATCAATATTCTGGAGGCGTTCGCAGCAGCGCCGCCACCGCTGGACTACGTTTTGCCCAACATGGTGGCCGGTACGGTCGGGG
CGCTGGTGTCGCCCGGTGGTGCCGGTAAATCCATGCTGGCCCTGCAACTGGCCGCACAGATTGCAGGCGGGCCGGATCTGCTGGAGGTGGGCGAACTGC
CCACCGGCCCGGTGATCTACCTGCCCGCCGAAGACCCGCCCACCGCCATTCATCACCGCCTGCACGCCCTTGGGGCGCACCTCAGCGCCGAGGAACGGCA
AGCCGTGGCTGACGGCCTGCTGATCCAGCCGCTGATCGGCAGCCTGCCCAACATCATGGCCCCGGAGTGGTTCGACGGCCTCAAGCGCGCCGCCGAGGG
CCGCCGCCTGATGGTGCTGGACACGCTGCGCCGGTTCCACATCGAGGAAGAAAACGCCAGCGGCCCCATGGCCCAGGTCATCGGTCGCATGGAGGCCAT
CGCCGCCGATACCGGGTGCTCTATCGTGTTCCTGCACCATGCCAGCAAGGGCGCGGCCATGATGGGCGCAGGCGACCAGCAGCAGGCCAGCCGGGGCA
GCTCGGTACTGGTCGATAACATCCGCTGGCAGTCCTACCTGTCGAGCATGACCAGCGCCGAGGCCGAGGAATGGGGTGTGGACGACGACCAGCGCCGGT
TCTTCGTCCGCTTCGGTGTGAGCAAGGCCAACTATGGCGCACCGTTCGCTGATCGGTGGTTCAGGCGGCATGACGGCGGGGTGCTCAAGCCCGCCGTGCT
GGAGAGGCAGCGCAAGAGCAAGGGGGTGCCCCGTGGTGAAGCCTAA

RSF1010 Backbone (continued); *Replication Protein C* (14 base overlap with *Replication Protein A*):
GTGGTGAAGCCTAAGAACAAGCACAGCCTCAGCCACGTCCGGCACGACCCGGCGCACTGTCTGGCCCCCGGCCTGTTCCGTGCCCTCAAGCGGGGCGAG
CGCAAGCGCAGCAAGCTGGACGTGACGTATGACTACGGCGACGGCAAGCGGATCGAGTTCAGCGGCCCGGAGCCGCTGGGCGCTGATGATCTGCGCAT
CCTGCAAGGGCTGGTGGCCATGGCTGGGCCTAATGGCCTAGTGCTTGGCCCGGAACCCAAGACCGAAGGCGGACGGCAGCTCCGGCTGTTCCTGGAACC
CAAGTGGGAGGCCGTCACCGCTGAATGCCATGTGGTCAAAGGTAGCTATCGGGCGCTGGCAAAGGAAATCGGGGCAGAGGTCGATAGTGGTGGGGCG
CTCAAGCACATACAGGACTGCATCGAGCGCCTTTGGAAGGTATCCATCATCGCCCAGAATGGCCGCAAGCGGCAGGGGTTTCGGCTGCTGTCGGAGTAC
GCCAGCGACGAGGCGGACGGGCGCCTGTACGTGGCCCTGAACCCCTTGATCGCGCAGGCCGTCATGGGTGGCGGCCAGCATGTGCGCATCAGCATGGA
CGAGGTGCGGGCGCTGGACAGCGAAACCGCCCGCCTGCTGCACCAGCGGCTGTGTGGCTGGATCGACCCCGGCAAAACCGGCAAGGCTTCCATAGATAC
CTTGTGCGGCTATGTCTGGCCGTCAGAGGCCAGTGGTTCGACCATGCGCAAGCGCCGCCAGCGGGTGCGCGAGGCGTTGCCGGAGCTGGTCGCGCTGG
GCTGGACGGTAACCGAGTTCGCGGCGGGCAAGTACGACATCACCCGGCCCAAGGCGGCAGGCTGA

RSF1010 Backbone (continued):
CCCCCCCCACTCTATTGTAAACAAGACATTTTTATCTTTTATATTCAATGGCTTATTTTCCTGCTAATTGGTAATACCATGAAAA
ATACCATGCTCAGAAAAGGC (Primer 149)
TTAACAATATTTTGAAAAATTGCCTACTGAGCGCTGCCGCACAGCTCCATAGGCCGCTTTCCTGGCTTTGCTTCCAGATGTATGCTCTTCTGCTCCTGC

Schizokinen Operon Complementation –*all0396* RSF1010 Plasmid (16,083 bp) [15]

This was used to create the FC–*all0396* complement mutant, via introduction into the knock out, but lacking the gene *all0396*; hence it contains genes *all0390* – *all0395*.

CGCCTATTATTATTGACTTGCATTAGCAATAGTTTTTGATTTTATTAAAATATATCTATTTCAGTACTTATACAAGTATTTTCTTGAAGAAGTATCTCAAAAAG
GTATGAATTAACTACGAAAAAACCAATGATAAAAGCCGATAAAGCTCTTCCTACATAGTCTCTAGATATTTTGCTTTTTGTCTATTTACTGTTAGGAATTATT
CTTGCTGACATTATCATTAATAACGCACACATACCTTTATAAGCGTGTTTTCGGTATCAGTTTCATTAGTATGATGATGCAACTAGCTTTAGATAAGAAGAA
TTAATGAATCAAAAATTTCTTATCTTTTTGTCAAGTTATTAGTAGACTTTTTCATTAATCTTCATGTAGGATGTTTAGCAGGTAGGAAATTAATGCAAGTAAT
TCTCAAATATTCTGTTCCCACTTACCATTAAGGCTCAACGCCTAGAGGATTTTCACA

All0395:
ATGGTGATTATTTCGCGCAGTTCGGCTTTGCCGTTCCCGCAGGGTGGCGCAAAGGAGGACACTTGCGTGGGCGGGTCTCCCGACTTGAGCAAAGTGTCCG
TCACGCAAAGAAAGAAGTTTGATGAGTTTTTTGTTGCAGGAGAGGGTTTGCGGGAGGCGATCGCTGCTGCTGGAGAGGTGTTGATTGATTATTTTGCTAC
TCAAGAAAAACCCTATAGTGGTAGGAGTCCCCAGGATTTAACTAGGACTATT GCAGATATCGCTGTGTGTCC (Primer 175 antisense)
TGATGAGGGGGTGGCTTTGAGTCAGGTTTTGGCTGAGGTGGGGGAGAATATCATCAAGCATTCGGTGGTGGTAACTCATCCTACTTGTATGGCTCATTTG
CATTGTCCACCGTTATTACCTGCTGTGGCGGCTGAGGTGTTGATTAGTGGGACTAATCAATCTCTGGATTCTTGGGATCAAAGTCCGGCTGCTACTGTTTTG
GAACAGCAGGTGGTAAATTGGCTGTGTGCAAGTTTTGGTTATGATGCTGATGCTGATGGTATATTTACCAGTGGCGGTACGCAATCGAATTTTATGGGGGT
TGCTCCTAGCGCGTGATGCTTACGCACGTCATCAGTTAAACTGGTCTGTGCAGCAGCAAGGATTACCACCAGAAGCTCAACGTTTTCGGATTCTCTGTTCTC
AAGCTGCTCATTTCACCATTAGTCAAGCTGCTTCTTTACTCGGTTTGGGACAGCAAGCTGTGGTAACGGTGGAGACTGATAGTGATTATCAGCTTTGTGCG
GCGGCGGTAGAACAGAAGTTAGAAGAATTGCAGCAACAGAATTTATTACCCATTGCTTTAGTTGCAACTGCGGGAACTACGGATTTTGCTAGTATTGACA
AATTACCAGAATTAGCCGCCTGTGCTGAGAAATATGGCCTGTGGTTTCATGTGGATGCGGCTTTTGGTGGTGCATTGGTGATGAGCGATCGCCATCGAGA
TAAACTAGATGGTATTGCTTTGGCTGACTCGATTACAGTCGATTTCCATAAACTGTTTTACCAGCCAATTAGCTGTGGTGCTTTTTTAGTTAAACAGCGTCAA
AACTTTGATTTAATAAAGCTACACGCTGATTATCTCAACCCTGAAACCAACGAAGTAGCCAGCATTCCCGACTTAGTAACTAAGTCCATACAAACCACCAAG
CGATTCGACGCGCTCAAATTGTTTGTTTCTCTCCGCACTCTGGGGAGGAAACAATTTGCACAGATGATTGATACAACAATTGAACTAGCCAAGGAAACCGC
TAGTTTAATTGACGCTGAACCTGCATTGGAGTTGGCAAACAATCCTACCATTAACGCTGTTGTTTTTCGCTATCTTCCTAGTGAAACACCAGCACACATAGA
TAGTACAACTTGGGCAAATCAAATCAATAGCCATATTCGCATGAGTTTACTGCAACAAGGTATCGCAGTCATCGCACAGACCAAAATTGGTCAACTTACCT

ATCTAAAGTTTACCTTGCTGAATCCTCAGACGGCGATCGCTGATATTCAAGAGGTTCTCAACTCTATCACAACGATAGGTGAGAAGTATTTATTTCACGCAC
AAGAGAGTAAAGAAGGGTTAAGCCTCTAA

GCTCGACACTCTCATTCTCTCCGCGTCTCTGCGTGAGCAAAAAATAGGAGAGAGGGAGAGCGAACAACTCTCCTCACTCCCAACATTAAATAACTTTCACC
CAAACAATACACTAGAC

All0394:
ATGACACCATACACAATTGCCAAACCAACCTCGGTGCTGGTGGAATATCGCACTGATAAACAAATTGCAGAACAAGCAACTATTCACAGCTTTTTGAATTG
CTATCTGCGCGAAACCAACACGGGTAAACTCATCACCACCGCGACAAAAGACGCTGATATTTTAGAAGTTTTCCAAAACACAAATACAAAATCGCTCATCT
GCTGTGAGTTAAAGCAACAAAATCTGAGATTATTAATCGGTTTAAGATATTACTCACCCACAGGTAGACATTTATTTGCTTTCCCCTTTATTATCAAGTAGA
TAAAGGTAATTTACTTGAACTGGATTATCTCACCTTAGCAACGCTCATTACTAAAGAATTATCCTTAGCTGGTGGTAGCAATAGCCATCAAGATGAACTGAT
TTTGCGAGTGATTCAAAGCTGCAATCACATTGAATATTTCGTTCAGAAACGGCGACAAGATATAGAAAAGCTCTATACTTTTAACAGTAATTTCATCGCATC
TGAACAAGCTTTAGTTTTTGGACACCATTTACACCCCACCCCAAAAAGTCGGCAAGGTTTTGCTGACCATGAATTGTCAATTTATTCGCCAGAATTAAAAGG
TAGTTTTCCTCTGCATTACTTCCGCATTCATCAGTCAATGGTACTGGAAGGTTCGCAGCTATCACAGACAGCAACAACGCTGATTAAATCAGAATTATTGGC
TGATCCAAAGGTTGATAATCAATTCAAAAATACTTACTGTAATGAAGATGAATATGCTTTATTACCGATACACCCTTGGCAAGCTAATTATTTACTGCAACA
GCCACAAATTCAACAATTAATTAAGCAAGAAATATTGCAAGATTTAGGTTTAGTCGGTCGAGCATATCAACCTACATCTTCAATTCGCACTGTCTATCATCC
AGATGCGGCATTTATGTTAAAATTGTCGCTGAATATCAAAATTACCAACTCTGTCCGCACTAATTTATATAAAGAATTAGAACGGAGTTTAGAAGTTCATCA
AATTTTAACAAGTGAAATTGGGCAACAACTTTATCAGCGTTTTCCTGAGTTCCAAATCATCACTGATCCTGCTTATATCACCTTAAAAATTGATGGTGTTGCT
GTTGATGGTTTCTCAACAATTCTGCGAGAAAATCCGTTTTTAAATAATCCCCAGACGGATGCAACTTGTGTAGTAGCTTTATGTCAAGACTCTATTTTGGGT
AACGGTTCACGATTAGCACGGATTATTGAAGAACTAGCACAACAAGAAAACCGTTCGACTGAGGCGGTGAGTTTGGATTGGTTCAACCGTTATTTACAGA
TTTACTTAGAGCCAATTCTCTGGTTATATTTCACTTACGGGATAGGACTAGAAGCCCATCAGCAAAATAGTGTTGTACAGTTAAAAAATGGCTATCCTGAG
AAGTTCTTTTATCGTGACAACCAAGGTTATTATTACCGTCGTTCTTGTCATCAATTGTTAGATAATATTTTGCCGGGGATTAGTCAAAAGAGTGAGACAATA
TGTGATGATGAGGTTATTGATGAACGATTGACTTACTACTTGTTTTTTAATAATTTGTTCGGGTTAATTAATGCTTTTGGTGTAGCTGGACTTGTAGATGAG
GAATTACTCTTAGG GGAGTTGCGAAACATCTTAGGC (Primer 62 antisense)
AAATATTCTGAACATTCTTTAGTGAATAATTTACTTTTTCAATCACAGTTACTTTGTAAGGCTAATCTTCTCACCCGGTTTCATAATCTGGATGAACTGGTGG
GGCCAGTTTCTACACAGTCTGTCTATGTTGCTGTTGATAATCCTTTGATGTAA

ACGCAGAGGGACGCAGGGGTTTACGCAAAGTAACGCAGAGAGTTTATGAGAGGAATTTGAGGCTGAAGTTT

All0393:
ATGTCGGTTAGTATGATTAATTACAGTTACGCAAGGTTTGATGCTGCAATCAATAAAACTATTGCTTTTCGTCCGGTGGTTTTGGAGGAGGATTTAAACCTG
ATTCATAACTGGATGAATCAACCTCATGTAATTCCTTTTTGGAATTTAGCCTTTGATTTGGAACGGATGCGGGAGCATTTACAAAGGACTTTAGCAGATAAA
CATCAAACTCTTTATATTGGTTGTTTAGATGATGAACCGATGAGTTATTGGGAGTCATACTGGACAATTGATGATATTGTGGCTCGACATTATTCAGCAGAA
GCGACAGATCAAGGTATTCATTTGTTAATTGGGGAAACCAAATTTTTAGGTAAAGGTTATGCTTTGCCACTGTTACGGGCAATGGTATTTTTTCAATTTGAA
AATACAGCAACTCAAAAGATTATTGCTGAACCAGATATTCGCAATCAAAAGATGATTCATGTTTTTGAAAAGTGTGGTTTTGAGTTTCAGAAAGAGATTGA
ATTACCGGATAAATTTGGGGCGTTGATGTTTTGCGATCGCCAGCTATTTTTTCAGAGGTGGAAAGCATGGTAA

All0392 (7 base overlap with *All0393*):
ATGGTAAATTGTGTTTATGACTTGATTGGTATTGGTCTTGGGCCTTTTAATTTAGGTTTAGCGGCGCTGTTAGAACCAATAACAGAGATTAAGTCTTTATTC
CTCGAACAAAAGCCTCAATTTCAATGGCATCCAGGGTTGTTACTTGAGGGGACGACAATTCAAGTACCATTTTTGGCAGACTTGGTGACAATGGCTGAACC
TAGTAGTAAATTCAGCTTTCTTAGTTACCTGAAAGCTAAGTCTCGTCTCTATAATTTTTACTTCTGGGAAGAGTTTCATATTCCCAGGCGAGAATATAACGAT
TACTGTCAATGGGTAGCAAAGCAATTACCAAATTGTTACTTTGGTGAACAGGTAAAAAGTATTGATTGGGATGAAAAAGCGCAGGAATTTATAGTGTCTG
GTACTAATTTCATCTACCGTTGTCGCAATTTGGTTTTAGGCGTTGGGACTGTTCCCTACATACCGCCTTGTTTCCGTGATTTAGTATCAGAAAATGTTTTTCA
CTCGTCCAAATTTCTCCATCAAAAAGTAAGCTGTCGTCAAGCGAAATCAATCACAGTTATCGGTTCTGGACAAAGTGCAGCCGAGGTTTTTTATGAACTATT
GCAAGAGCAAGAAAACTATGATTATCACCTAGAATGGCATACTCGTTCTTCCGGCTTTTTCCCAATGGAATATTCCAAATTGGGGTTAGAACATTTTTCACC
CGATTACATCCATTATTTCTACCATCTGCAACCAGAACAGCGAGACGAACTATTAACTAAACAAGGGTTGCTGTATAAGGGAATTAGCTTTAACACCATAG
CCAAAATATATGATTTGCTTTACGAGCGTTCCGTTGCTGATAATTACCCTGATGTTAAATTACTCTCTGGAGTAGAAGTTAAAGACATAGAACCCACTGCTG
AAGGTTATCGTCTCACCTATCGCCATTCTCATCAGCATCAACCATTTATTCATGAAAGCGATCGCATTATCTTAGCTACAGGCTATCATCACGCCACCCCTAA
TTTTATGGCAGATATCCGCGATTTGCTGCAATGGGATGAAAAAGGGCGTTACAAGGTAAATTTTGACTATCATCTCTCCCTGACTCAAGACATCCCCAACC
GGATTTTTGTCCAAAACGCTGAGTTACATACCCACGGGATTGGTGCGCCAGATTTGGGTTTAGGTTGTTACCGCAATTCTGTCATTATCAACTCCTTAACGG
GACGCAATACGTACCCAGTACAACAGCGCAACGTCTTTCAGCAATTTGGTCTAGTCCCATGA

All0391 (4 base overlap with *All0392*):
ATGAAGCATCGGCTACCCCTCATCTCAGGTGCATTATTTTTATGTGTGTTCTTGAGTATTTTCAACGAGGTTTTACTGTCACCCTTTTATCCCCAGTTTTTCCG
TAAAGTCTTTGGCGTGACAGATTTAGCCTACACCGGTTACTACATCTTTGTGTGTCGGTTAACCGTGGTGCTGTGTGCGCCTGTGTGGGGAGTGTTGTCAC
GCCGCTTTGAAGTCAAACACCTACTCTTTGTTGGACAATTGGGTGCAGCCTTCATGACAGCCTTGATGGGTACAAGCAGCAGTGTAGAGCAATTTCTGATG
TACACAATTTTGCTGTTACTGTGCAAAAGCAGTTATTTGCTAGTGTATCCCCTCATTATCCAACTAGGAGGTGAAGAAAAACGAGCTGCGATCGCCGGCAC
ATACCAAGCTGTATTTCATGGTGCAATTATCATCGCTACCATCGTCGGCGCATTTATGGTCAATATTGACACACCCTTAATTATTTTTTACGGGATTGCCGCA
GCCGATCTTTTACAACTTGCCATCTGTGCTTATATGTTGCGGGGTGTATCTACCGCAGGGGGGCAGGGAGCAGGGGGCAGGGGAGAAAATCAGCCAGTA
GCACCAAACCAACTGGGTTACATCATCGCTATCGGGGTAGTAATTCTCACCTTCCAACTAGCCAATAACTTAGTCCGTCCCTACTTCACAGCCTATGTTACC
GCCGAACCACTAAAAGTTGACCTGCTCACAAGTAGCTTGTTATTTCTGATACCCAGTGTAATGGCGATCGCTGCCTTACCCTATATTCGTCAAGCCTGTCGT
CCCGAACGCCTCCATACCATCTACTTAGGAAGTTTAAGCCTACTCATCGTCAGCTTAGGCATACAAGGATTATCATCCAACCTACCCTTACTCATCCTAGCGA
GGATAGTTTACGGCTTCTTCCTAGCAGTCACCCAAGCCGCCTTAGAACTGCAAATATTCAACAAAAGCACAGCCAAACACCTCCACTTCAACTACAGCCTTG
CCACCTCCTTCGCCAACATCGGACACTTAGGCGCACCCCTTTTAGCCTCCTGGCTAGTCAACACCCACAGCCTAGCCTCCCCATTCATCGCCGCCACCATAAT
TTGCTGTCTAAACCTCCTATTCTTTCGCTATGTTCCGAAGGCAGGCGGCAGGCGGCAGGCGGCAGAAGGATTTTAA

CTCTGTTTCTCTTAAATCTGATTTTTCACGTTATGTGGAAAAATCTACGCTTAACCTAACCCCCTAACCCCCTTCCCTAGTAGGGAAGGGGGAAAATTCAAA
GCCTCTCTCCGTGTCGGGGAGAGGTTTGGAGAGGGGTTATCCAGAACCCGTGAAAATTCCCAAACAAAAATATGCAAAACCTAACCAAAATCCTCCAATA
CCCACGCTGGCAAACAGTTAGCCAAAAACTCCTAGCCAAA

All0390:
ATGCTCTCCGAATTCATGTATGAAGAAATCATCAAACCGGAGACAATAGAACAAACAGCAGAATATACCCTCTACCACCTCGCCCTCCCCGAAGGCATCGC
CTACAACTTCCAAGCCAAAAAACGCCTATTCGACAGCTACCGCGTCATCCCCGCATCTATCCAACGACGAGAAGCGGGAGAATTTTCCCCAGCATTCAACC
CCCTGCAATTCGTCCTCGATATTCACACATTTGTAGGAATGACGGCTGAAACCACAGCTCATTTAATCAAAGAACTCAGCAACACCCTACTAGCCGACGCAC
ATATTCAAACCAAAAAAGAAACCCAAGATATAGACTTACTCAATTTAGATTACCCCTCATTAGAAGGGGAAATGGAAGGACACCCCTGGATAACCTTCAAT
AAAGGACGCATTGGTTTTGGCTATGACGATTACCTAGCCCACGCACCCGAAAGTAAACAGCCAGTTTCCCTCTTTTGGATTGCTGTTAGCAGTGAACGCGC
CCAATTTAACGCCATCCCAGGACTAGATTATGTCACCCTCATTCAAGAAGAATTAGGCGCAGAAAGTCTTGCGGAAATTTACGGCTATTTTAGAACAACGTC
ATTTCAGCCCCGCAGATTATTATTTTCTTCCCGTCCATGATTGGCAATGGAAAAATATCATCACCCTTTTGTTTGTGGAAGAAATAGCCACAGGTGGAATTA
TTCCCCTCGGCTACAGCCAAGATAAATATTTACCCCAACAATCGATTCGCACCTTTGCTAATATCAGCTATCCGCAGAAACGGTACGTTAAATTACCCCTGA
GCATTTTAAATACTCTCGTTTACCGTGGTTTACCAGGCGATCGCACACAGGTTGCCCCACTGGTTACAGAATATGTAAAATCGATTTGTGATCATGACCCTT
TCCTCAAAGATGAGTGTCGCTTAATTCTTCCTGGGGAAATTGCCAGCATCAACTACGATCATCCATACTACAGCCAGCTTTCAGGCGCACCTTACCAATATA
AGGAAATGTTGGGTTGTCTATGGCGCGAGAGTGTTTTAGCTTACACCAAAGCCGATGAACGTCCAATTACTTTAGCGTCTTTATTACATATCGATGGTAAC
GGTCAACCCTTTATTTCTCAACTGGTAGAACGTTCTGGACTCAGTTTAGATGAATGGTTATCTCGACTATTCAACACGATTTTACCGCCATTACTACATTACC
TCTACCGCTACGGCGTGGTTTTCTCCCCCCACGGTGAAAACACAATTTTGGTGCTGAAGGATTTTGCCCCCCATCGGTTAGCGATGAAAGATTTTGTCGATG
ATGTAAATATCAGTCGTCATCCCCTACCGGAATTAGAGACTTTAACACCACAACTCAAAGCCGTTTTGTTGACTGAACCACCAGAAGGATTATGTCAATTTA
TCTTTGCTGGCTTGTTTATCTGTCACCATCGTTATCTGTCTGATTTATTGGCAGACTACCACAACTACCCAGAACAGACCTTCTGGACAAAAGTCAGAGAGA
CGATTTTAAGCTATCAAAGTCGTTTCCCCGAAATGCAAGACAGATTTGAGTTATTCAACTTGCTAGCACCCCAATTTACCAAGCTGTGCTTGAATCGCAATC
GCCTGATTACTTACGGTTACGCCGATGATGGCGATCGCCCCCATGCGGCCGCCTTCGGTAAAGTGAATAATGCTTTGTATACAGTAGCCCAATTAATGCCA
ATGGAGCGCTAA

TAATAATTCGCTCGGTTGCCGCCGGGCGTTTTTTATTACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAA
GTAAACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGC

Neomycin Phosphotransferase II:
ATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGAT
GCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTCCAAGACGAGGCAGCGCGGC
TATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGC
AGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCG
ACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGC
CAGCCGAACTGTTCGCCAGGCTCAAGGCGCGGATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAA
ATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGG
CGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGA

GCGGGACTCTGGGGTTCGCTAGAGGATCGATCCTTTTTAACCCATCACATATACCTGCCGTTCACTATTATTTAGTGAAATGAGATATTATGATATTTTCTG
AATTGTGATTAAAAAGGCAACTTTATGCCCATGCAACAGAAACTATAA

RSF1010 Backbone:
CTGAAAGCGACCAGGTGCTCGGCGTGGCAAGACTCGCAGCGAACCCGTAGAAAGCCATGCTCCAGCCGCCCGCATTGGAGAAATTCTTCAAATTCCCGTT
GCACATAGCCCGGCAATTCCTTTCCCTGCTCTGCCATAAGCGCAGCGAATGCCGGGTAATACTCGTCAACGATCTGATAGAGAAGGGTTTGCTCGGGTCG
GTGGCTCTGGTAACGACCAGTATCCCGATCCCGGCTGGCCGTCCTGGCCGCCACATGAGGCATGTTCCGCGTCCTTGCAATACTGTGTTTACATACAGTCT
ATCGCTTAGCGGAAAGTTCTTTTACCCTCAGCCGAAATGCCTGCCGTTGCTAGACATTGCCAGCCAGTGCCCGTCACTCCCGTACTAACTGTCA

RSF1010 Backbone (continued); RSF1010 OriV Origin of Replication:
CGAACCCCTGCAATAACTGTCACGCCCCCCTGCAATAACTGTCACGAACCCCTGCAATAACTGTCACGCCCCCAAACCTGCAAACCCAGCAGGGGCGGGG
GCTGGCGGGGTGTTGGAAAAATCCATCCATGATTATCTAAGAATAATCCACTAGGCGCGGTTATCAGCGCCCTTGTGGGGCGCTGCTGCCCTTGCCCAAT
ATGCCCGGCCAGAGGCCGGATAGCTGGTCTATTCGCTGCGCTAGGCTACACACCGCCCCACCGCTGCGCGGCAGGGGGAAAGGCGGGCAAAGCCCGCT
AAACCCCACACCAAACCCCGCAGAAATACGCTGGAGCGCTTTTAGCCGCTTTAGCGGCCTTTCCCCCTACCCGAAGGGTGGGGGCGCGTGTGCAGCC

RSF1010 Backbone (continued): CCGCAGGGCCTGTCTCGGTCGATCAT

RSF1010 Backbone (continued); *Mobilisation Protein C* (antisense):
TCAGCCCGGCTCATCCTTCTGGCGTGGCGGCAGACCGAACAAGGCGCGGTCGTGGTCGCGTTCAAGGTACGCATCCATTGCCGCCATGAGCCGATCCTCC
GGCCACTCGCTGCTGTTCACCTTGGCCAAAATCATGGCCCCCACCAGCACCTTGCGCCTTGTTTCGTTCTTGCGCTCTTGCTGCTGTTCCCTTGCCCGCACCC
GCTGAATTTCGGCATTGATTCGCGCTCGTTGTTCTTCGAGCTTGGCCAGCCGATCCGCCGCCTTGTTGCTCCCCTTAACCAT

RSF1010 Backbone (continued):
CTTGACACCCCATTGTTAATGTGCTGTCTCGTAGGCTATCATGGAGGCACAGCGGCGGCAATCCCGACCCTACTTTGTAGGGGAGGGCGCACTTACCGGTT
TCTCTTCGAGAAACTGGCCTAACGGCCACCCTTCGGGCGGTGCGCTCTCCGAGGGCCATTGCATGGAGCCGAAAAGCAAAAGCAACAGCGAGGCAGC

RSF1010 Backbone (continued); *Mobilisation Protein A*:
ATGGCGATTTATCACCTTACGGCGAAAACCGGCAGCAGGTCGGGCGGCCAATCGGCCAGGGCCAAGGCCGACTACATCCAGCGCGAAGGCAAGTATGCC
CGCGACATGGATGAAGTCTTGCACGCCGAATCCGGGCACATGCCGGAGTTCGTCGAGCGGCCCGCCGACTACTGGGATGCTGCCGACCTGTATGAACGC
GCCAATGGGCGGCTGTTCAAGGAGGTCGAATTTGCCCTGCCGGTCGAGCTGACCCTCGACCAGCAGAAGGCGCTGGCGTCCGAGTTCGCCCAGCACCTG
ACCGGTGCCGAGCGCCTGCCGTATACGCTGGCCATCCATGCCGGTGGCGGCGAGAACCCGCACTGCCACCTGATGATCTCCGAGCGGATCAATGACGGC

ATCGAGCGGCCCGCCGCTCAGTGGTTCAAGCGGTACAACGGCAAGACCCCGGAGAAGGGCGGGGCACAGAAGACCGAAGCGCTCAAGCCCAAGGCATG
GCTTGAGCAGACCCGCGAGGCATGGGCCGACCATGCCAACCGGGCATTAGAGCGGGCTGGCCACGACGCCCGCATTGACCACAGAACACTTGAGGCGC
AGGGCATCGAGCGCCTGCCCGGTGTTCACCTGGGGCCGAACGTGGTGGAGATGGAAGGCCGGGGCATCCGCACCGACCGGGCAGACGTGGCCCTGAAC
ATCGACACCGCCAACGCCCAGATCATCGACTTACAGGAATACCGGGAGGCAATAGACC

RSF1010 Backbone (continued); *Mobilisation Protein A* (continued); *Mobilisation Protein B*:
ATGAACGCAATCGACAGAGTGAAGAAATCCAGAGGCATCAACGAGTTAGCGGAGCAGATCGAACCGCTGGCCCAGAGCATGGCGACACTGGCCGACGA
AGCCCGGCAGGTCATGAGCCAGACCCAGCAGGCCAGCGAGGCGCAGGCGGCGGAGTGGCTGAAAGCCCAGCGCCAGACAGGGGCGGCATGGGTGGA
GCTGGCCAAAGAGTTGCGGGAGGTAGCCGCCGAGGTGAGCAGCGCCGCGCAGAGCGCCCGGAGCGCGTCGCGGGGGTGGCACTGGAAGCTATGGCTA
ACCGTGATGCTGGCTTCCATGATGCCTACGGTGGTGCTGCTGATCGCATCGTTGCTCTTGCTCGACCTGACGCCACTGACAACCGAGGACGGCTCGATCTG
GCTGCGCTTGGTGGCCCGATGA

RSF1010 Backbone (continued); *Mobilisation Protein A* (continued); *Replication Protein B* (3 base overlap with *Mobilisation Protein B*):
ATGAAGAACGACAGGACTTTGCAGGCCATAGGCCGACAGCTCAAGGCCATGGGCTGTGAGCGCTTCGATATCGGCGTCAGGGACGCCACCACCGGCCAG
ATGATGAACCGGGAATGGTCAGCCGCCGAAGTGCTCCAGAACACGCCATGGCTCAAGCGGATGAATGCCCAGGGCAATGACGTGTATATCAGGCCCGCC
GAGCAGGAGCGGCATGGTCTGGTGCTGGTGGACGACCTCAGCGAGTTTGACCTGGATGACATGAAAGCCGAGGGCCGGGAGCCTGCCCTGGTAGTGGA
AACCAGCCCGAAGAACTATCAGGCATGGGTCAAGGTGGCCGACGCCGCAGGCGGTGAACTTCGGGGGCAGATTGCCCGGACGCTGGCCAGCGAGTACG
ACGCCGACCCGGCCAGCGCCGACAGCCGCCACTATGGCCGCTTGGCGGGCTTCACCAACCGCAAGGACAAGCACACCACCCGCGCCGGTTATCAGCCGT
GGGTGCTGCTGCGTGAATCCAAGGGCAAGACCGCCACCGCTGGCCCGGCGCTGGTGCAGCAGGCTGGCCAGCAGATCGAGCAGGCCCAGCGGCAGCAG
GAGAAGGCCCGCAGGCTGGCCAGCCTCGAACTGCCCGAGCGGCAGCTTAGCCGCCACCGGCGCACGGCGCTGGACGAGTACCGCAGCGAGATGGCCGG
GCTGGTCAAGCGCTTCGGTGATGACCTCAGCAAGTGCGACTTTATCGCCGCGCAGAAGCTGGCCAGCCGGGGCCGCAGTGCCGAGGAAATCGGCAAGG
CCATGGCCGAGGCCAGCCCAGCGCTGGCAGAGCGCAAGCCCGGCCACGAAGCGGATTACATCGAGCGCACCGTCAGCAAGGTCATGGGTCTGCCCAGC
GTCCAGCTTGCGCGGGCCGAGCTGGCACGGGCACCGGCACCCCGCCAGCGAGGCATGGACAGGGGCGGGCCAGATTTCAGCATGTAG

RSF1010 Backbone (continued):
TGCTTGCGTTGGTACTCACGCCTGTTATACTATGAGTACTCACGCACAGAAGGGGGTTTTATGGAATACGAAAAAAGCGCTTCAGGGTCGGTCTACCTGAT
CAAAAGTGACAAGGGCTATTGGTTGCCCGGTGGCTTTGGTTATACGTCAAACAAGGCCGAGGCTGGCCGCTTTTCAGTCGCTGATATGGCCAGCCTTAAC
CTTGACGGCTGCACCTTGTCCTTGTTCCGCGAAGACAAGCCTTTCGGCCCCGGCAAGTTTCTCGGTGACTGATATGAAAGACCAAAAGGACAAGCAGACC
GGCGACCTGCTGGCCAGCCCTGACGCTGTACGCCAAGCGCGATATGCCGAGCGCATGAAGGCCAAAGGGATGCGTCAGCGCAAGTTCTGGCTGACCGAC
GACGAATACGAGGCGCTGCGCGAGTGCCTGGAAGAACTCAGAGCGGCGCAGGGCGGGGGTAGTGACCCCGCCAGCGCCTAACCACCAACTGCCTGCAA
AGGAGGCAATCA

RSF1010 Backbone (continued); *Replication Protein A*:
ATGGCTACCCATAAGCCTATCAATATTCTGGAGGCGTTCGCAGCAGCGCCGCCACCGCTGGACTACGTTTTGCCCAACATGGTGGCCGGTACGGTCGGGG
CGCTGGTGTCGCCCGGTGGTGCCGGTAAATCCATGCTGGCCCTGCAACTGGCCGCACAGATTGCAGGCGGGCCGGATCTGCTGGAGGTGGGCGAACTGC
CCACCGGCCCGGTGATCTACCTGCCCGCCGAAGACCCGCCCACCGCCATTCATCACCGCCTGCACGCCCTTGGGGCGCACCTCAGCGCCGAGGAACGGCA
AGCCGTGGCTGACGGCCTGCTGATCCAGCCGCTGATCGGCAGCCTGCCCAACATCATGGCCCCGGAGTGGTTCGACGGCCTCAAGCGCGCCGCCGAGGG
CCGCCGCCTGATGGTGCTGGACACGCTGCGCCGGTTCCACATCGAGGAAGAAAACGCCAGCGGCCCCATGGCCCAGGTCATCGGTCGCATGGAGGCCAT
CGCCGCCGATACCGGGTGCTCTATCGTGTTCCTGCACCATGCCAGCAAGGGCGCGGCCATGATGGGCGCAGGCGACCAGCAGCAGGCCAGCCGGGGCA
GCTCGGTACTGGTCGATAACATCCGCTGGCAGTCCTACCTGTCGAGCATGACCAGCGCCGAGGCCGAGGAATGGGGTGTGGACGACGACCAGCGCCGGT
TCTTCGTCCGCTTCGGTGTGAGCAAGGCCAACTATGGCGCACCGTTCGCTGATCGGTGGTTCAGGCGGCATGACGGCGGGGTGCTCAAGCCCGCCGTGCT
GGAGAGGCAGCGCAAGAGCAAGGGGGTGCCCCGTGGTGAAGCCTAA

RSF1010 Backbone (continued); *Replication Protein C* (14 base overlap with *Replication Protein A*):
GTGGTGAAGCCTAAGAACAAGCACAGCCTCAGCCACGTCCGGCACGACCCGGCGCACTGTCTGGCCCCCGGCCTGTTCCGTGCCCTCAAGCGGGGCGAG
CGCAAGCGCAGCAAGCTGGACGTGACGTATGACTACGGCGACGGCAAGCGGATCGAGTTCAGCGGCCCGGAGCCGCTGGGCGCTGATGATCTGCGCAT
CCTGCAAGGGCTGGTGGCCATGGCTGGGCCTAATGGCCTAGTGCTTGGCCCGGAACCCAAGACCGAAGGCGGACGGCAGCTCCGGCTGTTCCTGGAACC
CAAGTGGGAGGCCGTCACCGCTGAATGCCATGTGGTCAAAGGTAGCTATCGGGCGCTGGCAAAGGAAATCGGGGCAGAGGTCGATAGTGGTGGGGCG
CTCAAGCACATACAGGACTGCATCGAGCGCCTTTGGAAGGTATCCATCATCGCCCAGAATGGCCGCAAGCGGCAGGGGTTTCGGCTGCTGTCGGAGTAC
GCCAGCGACGAGGCGGACGGGCGCCTGTACGTGGCCCTGAACCCCTTGATCGCGCAGGCCGTCATGGGTGGCGGCCAGCATGTGCGCATCAGCATGGA
CGAGGTGCGGGCGCTGGACAGCGAAACCGCCCGCCTGCTGCACCAGCGGCTGTGTGGCTGGATCGACCCCGGCAAAACCGGCAAGGCTTCCATAGATAC
CTTGTGCGGCTATGTCTGGCCGTCAGAGGCCAGTGGTTCGACCATGCGCAAGCGCCGCCAGCGGGTGCGCGAGGCGTTGCCGGAGCTGGTCGCGCTGG
GCTGGACGGTAACCGAGTTCGCGGCGGGCAAGTACGACATCACCCGGCCCAAGGCGGCAGGCTGA

RSF1010 Backbone (continued):
CCCCCCCCACTCTATTGTAAACAAGACATTTTTATCTTTTATATTCAATGGCTTATTTTCCTGCTAATTGGTAATACCATGAAAA
ATACCATGCTCAGAAAAGGC (Primer 149)
TTAACAATATTTTGAAAAATTGCCTACTGAGCGCTGCCGCACAGCTCCATAGGCCGCTTTCCTGGCTTTGCTTCCAGATGTATGCTCTTCTGCTCCTGC

Schizokinen Operon Complementation –*all0395* RSF1010 Plasmid (15,948 bp) [15]

This was used to create the FC–*all0395* complement mutant, via introduction into the knock out, but lacking the gene *all0396*; hence it contains genes *all0390* – *all0394* and *all0396*.

CGCCTATTATTATTGACTTGCATTAGCAATAGTTTTTGATTTTATTAAAATATATCTATTTCAGTACTTATACAAGTATTTTCTTGAAGAAGTATCTCAAAAAG
GTATGAATTAACTACGAAAAAACCAATGATAAAAGCCGATAAAGCTCTTCCTACATAGTCTCTAGATATTTTGCTTTTTGTCTATTTACTGTTAGGAATTATT
CTTGCTGACATTATCATTAATAACGCACACATACCTTTATAAGCGTGTTTTCGGTATCAGTTTCATTAGTATGATGATGCAACTAGCTTTAGATAAGAAGAA
TTAATGAATCAAAAATTTCTTATCTTTTTGTCAAGTTATTAGTAGACTTTTTCATTAATCTTCATGTAGGATGTTTAGCAGGTAGGAAATTAATGCAAGTAAT
TCTCAAATATTCTGTTCCCACTTACCATTAAGGCTCAACGCCTAGAGGATTTTCACA

All0396:
ATGCAACTCAATCACAATGGCGTACACTCTGAAGGCCACTCTTTCGTGCTGCCGCAGATATGTCCATCGCAGAGTAGTGTAGAGTCTGATTTTCCCAGTAA
TCGCGTGCGTTCTGTACCTGGTGCGGCATCAAGCCACTATTTAGAGCGTCAGCAAGCACGAGAGTCCAACGCTAGAAGCTATCCCCGGCGGATTCGGATT
GCCATTAGTGAGGCTCAGGGTATTTATCTCAAAGATGCTGATGGCAATGTATATATTGATTGTTTGGCTGGTGCAGGTACTTTAGCATTAGGACACAATCA
CCCAGTAGCAATAGAAGCGATGCGCCGGGTGCTAGATACGGGTTTACCTTTGCATACTCTAGATTTGACTACACCAGTTAAAGATCAGTTTGTCGAAGAAA
TTTTTGCTAGTTTACCTGCGGAATTTGCTCAGAATGCCAAGATTCAGTTTTGTGGGCCTTCGGGAGCAGATGCTGTAGAGGCGGCGATTAAACTGGTGAAA
ACTGCTACTGGCCACCGCAGTGTGTTGTCATTTCATGGTGGTTATCATGGGATGACTCATGGAGCATTGAGTTTAACAGGCAATCTCAATCCTAAACAAGC
GGTGACAGGGTTAATGCCAGATGTCCACTTTTTGCCTTATCCCTATCATTATCGTTGTCCCTTTGGTTTGGGTGGAGAAGCTGGACAAATTACCAGCAGCCG
TTATATAGAATCGATTTTGGATGATCCTGAAAGTGGGATTGTCACACCGGCGGCGATGATTTTGGAAGTTGTGCAAGGGGAAGGTGGGGTGATTCCTGCA
CCTGATGATTGGTTGCGAGAGATGCGCCGGATTACCCGCGATCGCCATATTCCCCTCATTGTTGATGAGATTCAAACAGGCTTAGGACGGACTGGTAAACT
CTACGCTTTTGAGCATTCAGGGATTGTGCCAGATGTGTTGTTACTCTCCAAAGCTATTGGTGGGAGTTTACCTTTATCGGTGGTTTTGTACAACAAGGCACT
AGATAAATGGAGTCCAGGCGCACACGCAGGGACATTCCGGGGTAATCAAATGGCAATGGCAGCCGGAACAGCGACACTACAGTATATTTTAGAAAATTC
CTTGACTGAACACGCGGCAGCGATGGGCGATCGCTTGTTGAAACATCTGCACCAAATTCAAGGGGAAACTTACTGTATTGGAGAAGTGCGGGGACGCGG
TTTGATGGTTGGTGTGGAGATTATTAATCCCCAAGCATCGGCTGATCGGCGTGGGAAGTATCCTGTACATCCGCAGTTGGCTAGTTGTATTCAGGCTGAGT
GTTTGCGTCGGGGTTTGATTGTGGAATTAGGGGGTAGATTTGGTAGCGTGGTGCGCTTTTTGCCGCC TTTGATTGTGACTCCAGCGC (Primer 176)
AGATTGATAGTATTAGTGAGATTTTTTCGTCGGCGGTACAGGCGGCGGAGAAGCAGGTTTTATCGGTTCTTTCGCAGGTTTCTTGA

GCTCGACACTCTCATTCTCTCCGCGTCTCTGCGTGAGCAAAAAATAGGAGAGAGGGAGAGCGAACAACTCTCCTCACTCCCAACATTAAATAACTTTCACC
CAAACAATACACTAGAC

All0394:
ATGACACCATACACAATTGCCAAACCAACCTCGGTGCTGGTGGAATATCGCACTGATAAACAAATTGCAGAACAAGCAACTATTCACAGCTTTTTGAATTG
CTATCTGCGCGAAACCAACACGGGTAAACTCATCACCACCGCGACAAAAGACGCTGATATTTTAGAAGTTTTCCAAAACACAAATACAAAATCGCTCATCT
GCTGTGAGTTAAAGCAACAAAATCTGAGATTATTAATCGGTTTAAGATATTACTCACCCACAGGTAGACATTTATTTGCTTTTCCCCTTTATTATCAAGTAGA
TAAAGGTAATTTACTTGAACTGGATTATCTCACCTTAGCAACGCTCATTACTAAAGAATTATCCTTAGCTGGTGGTAGCAATAGCCATCAAGATGAACTGAT
TTTGCGAGTGATTCAAAGCTGCAATCACATTGAATATTTCGTTCAGAAACGGCGACAAGATATAGAAAAGCTCTATACTTTTAACAGTAATTTCATCGCATC
TGAACAAGCTTTAGTTTTTGGACACCATTTACACCCCACCCCAAAAAGTCGGCAAGGTTTTGCTGACCATGAATTGTCAATTTATTCGCCAGAATTAAAAGG
TAGTTTTCCTCTGCATTACTTCCGCATTCATCAGTCAATGGTACTGGAAGGTTCGCAGCTATCACAGACAGCAACAACGCTGATTAAATCAGAATTATTGGC
TGATCCAAAGGTTGATAATCAATTCAAAAATACTTACTGTAATGAAGATGAATATGCTTTATTACCGATACACCCTTGGCAAGCTAATTATTTACTGCAACA
GCCACAAATTCAACAATTAATTAAGCAAGAAATATTGCAAGATTTAGGTTTAGTCGGTCGAGCATATCAACCTACATCTTCAATTCGCACTGTCTATCATCC
AGATGCGGCATTTATGTTAAAATTGTCGCTGAATATCAAAATTACCAACTCTGTCCGCACTAATTTATATAAAGAATTAGAACGGAGTTTAGAAGTTCATCA
AATTTTAACAAGTGAAATTGGGCAACAACTTTATCAGCGTTTTCCTGAGTTCCAAATCATCACTGATCCTGCTTATATCACCTTAAAAATTGATGGTGTTGCT
GTTGATGGTTTCTCAACAATTCTGCGAGAAAATCCGTTTTTAAATAATCCCCAGACGGATGCAACTTGTGTAGTAGCTTTATGTCAAGACTCTATTTTGGGT
AACGGTTCACGATTAGCACGGATTATTGAAGAACTAGCACAACAAGAAAACCGTTCGACTGAGGCGGTGAGTTTGGATTGGTTCAACCGTTATTTACAGA
TTTACTTAGAGCCAATTCTCTGGTTATATTTCACTTACGGGATAGGACTAGAAGCCCATCAGCAAAATAGTGTTGTACAGTTAAAAAATGGCTATCCTGAG
AAGTTCTTTTATCGTGACAACCAAGGTTATTATTACCGTCGTTCTTGTCATCAATTGTTAGATAATATTTTGCCGGGGATTAGTCAAAAGAGTGAGACAATA
TGTGATGATGAGGTTATTGATGAACGATTGACTTACTACTTGTTTTTTAATAATTTGTTCGGGTTAATTAATGCTTTTGGTGTAGCTGGACTTGTAGATGAG
GAATTACTCTTAGG GGAGTTGCGAAACATCTTAGGC (Primer 62 antisense)
AAATATTCTGAACATTCTTTAGTGAATAATTTACTTTTCAATCACAGTTACTTTGTAAGGCTAATCTTCTCACCCGGTTTCATAATCTGGATGAACTGGTGG
GGCCAGTTTCTACACAGTCTGTCTATGTTGCTGTTGATAATCCTTTGATGTAA

ACGCAGAGGGACGCAGGGGTTTACGCAAAGTAACGCAGAGAGTTTATGAGAGGAATTTGAGGCTGAAGTTT

All0393:
ATGTCGGTTAGTATGATTAATTACAGTTACGCAAGGTTTGATGCTGCAATCAATAAAACTATTGCTTTTCGTCCGGTGGTTTTGGAGGAGGATTTAAACCTG
ATTCATAACTGGATGAATCAACCTCATGTAATTCCTTTTTGGAATTTAGCCTTTGATTTGGAACGGATGCGGGAGCATTTACAAAGGACTTTAGCAGATAAA
CATCAAACTCTTTATATTGGTTGTTTAGATGATGAACCGATGAGTTATTGGGAGTCATACTGGACAATTGATGATATTGTGGCTCGACATTATTCAGCAGAA
GCGACAGATCAAGGTATTCATTTGTTAATTGGGGAAACCAAATTTTTAGGTAAAGGTTATGCTTTGCCACTGTTACGGGCAATGGTATTTTTTCAATTTGAA
AATACAGCAACTCAAAAGATTATTGCTGAACCAGATATTCGCAATCAAAAGATGATTCATGTTTTTGAAAAGTGTGGTTTTGAGTTTCAGAAAGAGATTGA
ATTACCGGATAAATTTGGGGCGTTGATGTTTTGCGATCGCCAGCTATTTTTTCAGAGGTGGAAAGCATGGTAA

All0392 (7 base overlap with *All0393*):
ATGGTAAATTGTGTTTATGACTTGATTGGTATTGGTCTTGGGCCTTTTAATTTAGGTTTAGCGGCGCTGTTAGAACCAATAACAGAGATTAAGTCTTTATTC
CTCGAACAAAAGCCTCAATTTCAATGGCATCCAGGGTTGTTACTTGAGGGGACGACAATTCAAGTACCATTTTTGGCAGACTTGGTGACAATGGCTGAACC
TAGTAGTAAATTCAGCTTTCTTAGTTACCTGAAAGCTAAGTCTCGTCTCTATAATTTTTACTTCTGGGAAGAGTTTCATATTCCCAGGCGAGAATATAACGAT
TACTGTCAATGGGTAGCAAAGCAATTACCAAATTGTTACTTTGGTGAACAGGTAAAAAGTATTGATTGGGATGAAAAAGCGCAGGAATTTATAGTGTCTG

GTACTAATTTCATCTACCGTTGTCGCAATTTGGTTTTAGGCGTTGGGACTGTTCCCTACATACCGCCTTGTTTCCGTGATTTAGTATCAGAAAATGTTTTTCA
CTCGTCCAAATTTCTCCATCAAAAAGTAAGCTGTCGTCAAGCGAAATCAATCACAGTTATCGGTTCTGGACAAAGTGCAGCCGAGGTTTTTTATGAACTATT
GCAAGAGCAAGAAAACTATGATTATCACCTAGAATGGCATACTCGTTCTTCCGGCTTTTTCCCAATGGAATATTCCAAATTGGGGTTAGAACATTTTTCACC
CGATTACATCCATTATTTCTACCATCTGCAACCAGAACAGCGAGACGAACTATTAACTAAACAAGGGTTGCTGTATAAGGGAATTAGCTTTAACACCATAG
CCAAAATATATGATTTGCTTTACGAGCGTTCCGTTGCTGATAATTACCCTGATGTTAAATTACTCTCTGGAGTAGAAGTTAAAGACATAGAACCCACTGCTG
AAGGTTATCGTCTCACCTATCGCCATTCTCATCAGCATCAACCATTTATTCATGAAAGCGATCGCATTATCTTAGCTACAGGCTATCATCACGCCACCCCTAA
TTTTATGGCAGATATCCGCGATTTGCTGCAATGGGATGAAAAAGGGCGTTACAAGGTAAATTTTGACTATCATCTCTCCCTGACTCAAGACATCCCCAACC
GGATTTTTGTCCAAAACGCTGAGTTACATACCCACGGGATTGGTGCGCCAGATTTGGGTTTAGGTTGTTACCGCAATTCTGTCATTATCAACTCCTTAACGG
GACGCAATACGTACCCAGTACAACAGCGCAACGTCTTTCAGCAATTTGGTCTAGTCCCATGA

All0391 (4 base overlap with *All0392*):
ATGAAGCATCGGCTACCCCTCATCTCAGGTGCATTATTTTTATGTGTGTTCTTGAGTATTTTCAACGAGGTTTTACTGTCACCCTTTTATCCCCAGTTTTTCCG
TAAAGTCTTTGGCGTGACAGATTTAGCCTACACCGGTTACTACATCTTTGTGTGTCGGTTAACCGTGGTGCTGTGTGCGCCTGTGTGGGGAGTGTTGTCAC
GCCGCTTTGAAGTCAAACACCTACTCTTTGTTGGACAATTGGGTGCAGCCTTCATGACAGCCTTGATGGGTACAAGCAGCAGTGTAGAGCAATTTCTGATG
TACACAATTTTGCTGTTACTGTGCAAAAGCAGTTATTTGCTAGTGTATCCCCTCATTATCCAACTAGGAGGTGAAGAAAAACGAGCTGCGATCGCCGGCAC
ATACCAAGCTGTATTTCATGGTGCAATTATCATCGCTACCATCGTCGGCGCATTTATGGTCAATATTGACACACCCTTAATTATTTTTTACGGGATTGCCGCA
GCCGATCTTTTACAACTTGCCATCTGTGCTTATATGTTGCGGGGTGTATCTACCGCAGGGGGGCAGGGAGCAGGGGGCAGGGGAGAAAATCAGCCAGTA
GCACCAAACCAACTGGGTTACATCATCGCTATCGGGGTAGTAATTCTCACCTTCCAACTAGCCAATAACTTAGTCCGTCCCTACTTCACAGCCTATGTTACC
GCCGAACCACTAAAAGTTGACCTGCTCACAAGTAGCTTGTTATTTCTGATACCCAGTGTAATGGCGATCGCTGCCTTACCCTATATTCGTCAAGCCTGTCGT
CCCGAACGCCTCCATACCATCTACTTAGGAAGTTTAAGCCTACTCATCGTCAGCTTAGGCATACAAGGATTATCATCCAACCTACCCTTACTCATCCTAGCGA
GGATAGTTTACGGCTTCTTCCTAGCAGTCACCCAAGCCGCCTTAGAACTGCAAATATTCAACAAAAGCACAGCCAAACACCTCCACTTCAACTACAGCCTTG
CCACCTCCTTCGCCAACATCGGACACTTAGGCGCACCCCTTTTAGCCTCCTGGCTAGTCAACACCCACAGCCTAGCCTCCCCATTCATCGCCGCCACCATAAT
TTGCTGTCTAAACCTCCTATTCTTTCGCTATGTTCCGAAGGCAGGCGGCAGGCGGCAGGCGGCAGAAGGATTTTAA

CTCTGTTTCTCTTAAATCTGATTTTTCACGTTATGTGGAAAAATCTACGCTTAACCTAACCCCCTAACCCCCTTCCCTAGTAGGGAAGGGGGAAAATTCAAA
GCCTCTCTCCGTGTCGGGGAGAGGTTTGGAGAGGGGTTATCCAGAACCCGTGAAAATTCCCAAACAAAAATATGCAAAACCTAACCAAAATCCTCCAATA
CCCACGCTGGCAAACAGTTAGCCAAAAACTCCTAGCCAAA

All0390:
ATGCTCTCCGAATTCATGTATGAAGAAATCATCAAACCGGAGACAATAGAACAAACAGCAGAATATACCCTCTACCACCTCGCCCTCCCCGAAGGCATCGC
CTACAACTTCCAAGCCAAAAAACGCCTATTCGACAGCTACCGCGTCATCCCCGCATCTATCCAACGACGAGAAGCGGGAGAATTTTCCCCAGCATTCAACC
CCCTGCAATTCGTCCTCGATATTCACACATTTGTAGGAATGACGGCTGAAACCACAGCTCATTTAATCAAAGAACTCAGCAACACCCTACTAGCCGACGCAC
ATATTCAAACCAAAAAAGAAACCCAAGATATAGACTTACTCAATTTAGATTACCCCTCATTAGAAGGGGAAATGGAAGGACACCCCTGGATAACCTTCAAT
AAAGGACGCATTGGTTTTGGCTATGACGATTACCTAGCCCACGCACCCGAAAGTAAACAGCCAGTTTCCCTCTTTTGGATTGCTGTTAGCAGTGAACGCGC
CCAATTTAACGCCATCCCAGGACTAGATTATGTCACCCTCATTCAAGAAGAATTAGGCGCAGAAAGTCTTGCGGAATTTACGGCTATTTTAGAACAACGTC
ATTTCAGCCCCGCAGATTATTATTTTCTTCCCGTCCATGATTGGCAATGGAAAAATATCATCACCCTTTTGTTTGTGGAAGAAATAGCCACAGGTGGAATTA
TTCCCCTCGGCTACAGCCAAGATAAATATTTACCCCAACAATCGATTCGCACCTTTGCTAATATCAGCTATCCGCAGAAACGGTACGTTAAATTACCCCTGA
GCATTTTAAATACTCTCGTTTACCGTGGTTTACCAGGCGATCGCACACAGGTTGCCCCACTGGTTACAGAATATGTAAAATCGATTTGTGATCATGACCCTT
TCCTCAAAGATGAGTGTCGCTTAATTCTTCCTGGGGAAATTGCCAGCATCAACTACGATCATCCATACTACAGCCAGCTTTCAGGCGCACCTTACCAATATA
AGGAAATGTTGGGTTGTCTATGGCGCGAGAGTGTTTTAGCTTACACCAAAGCCGATGAACGTCCAATTACTTTAGCGTCTTTATTACATATCGATGGTAAC
GGTCAACCCTTTATTTCTCAACTGGTAGAACGTTCTGGACTCAGTTTAGATGAATGGTTATCTCGACTATTCAACACGATTTTACCGCCATTACTACATTACC
TCTACCGCTACGGCGTGGTTTTCTCCCCCCACGGTGAAAACACAATTTTGGTGCTGAAGGATTTTGCCCCCCATCGGTTAGCGATGAAAGATTTTGTCGATG
ATGTAAATATCAGTCGTCATCCCCTACCGGAATTAGAGACTTTAACACCACAACTCAAAGCCGTTTTGTTGACTGAACCACCAGAAGGATTATGTCAATTTA
TCTTTGCTGGCTTGTTTATCTGTCACCATCGTTATCTGTCTGATTTATTGGCAGACTACCACAACTACCCAGAACAGACCTTCTGGACAAAAGTCAGAGAGA
CGATTTTAAGCTATCAAAGTCGTTTCCCCGAAATGCAAGACAGATTTGAGTTATTCAACTTGCTAGCACCCCAATTTACCAAGCTGTGCTTGAATCGCAATC
GCCTGATTACTTACGGTTACGCCGATGATGGCGATCGCCCCCATGCGGCCGCCTTCGGTAAAGTGAATAATGCTTTGTATACAGTAGCCCAATTAATGCCA
ATGGAGCGCTAA

TAATAATTCGCTCGGTTGCCGCCGGGCGTTTTTTATTACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAA
GTAAACTGGATGGCTTTCTTGCCGCCAAGGATCTGATGGCGCAGGGGATCAAGATCTGATCAAGAGACAGGATGAGGATCGTTTCGC

Neomycin Phosphotransferase II:
ATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGAT
GCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTCCAAGACGAGGCAGCGCGGC
TATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGC
AGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCCATTCG
ACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGC
CAGCCGAACTGTTCGCCAGGCTCAAGGCGCGGATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAA
ATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGG
CGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGA

GCGGGACTCTGGGGTTCGCTAGAGGATCGATCCTTTTTAACCCATCACATATACCTGCCGTTCACTATTATTTAGTGAAATGAGATATTATGATATTTTCTG
AATTGTGATTAAAAAGGCAACTTTATGCCCATGCAACAGAAACTATAA

RSF1010 Backbone:
CTGAAAGCGACCAGGTGCTCGGCGTGGCAAGACTCGCAGCGAACCCGTAGAAAGCCATGCTCCAGCCGCCCGCATTGGAGAAATTCTTCAAATTCCCGTT
GCACATAGCCCGGCAATTCCTTTCCCTGCTCTGCCATAAGCGCAGCGAATGCCGGGTAATACTCGTCAACGATCTGATAGAGAAGGGTTTGCTCGGGTCG

GTGGCTCTGGTAACGACCAGTATCCCGATCCCGGCTGGCCGTCCTGGCCGCCACATGAGGCATGTTCCGCGTCCTTGCAATACTGTGTTTACATACAGTCT
ATCGCTTAGCGGAAAGTTCTTTTACCCTCAGCCGAAATGCCTGCCGTTGCTAGACATTGCCAGCCAGTGCCCGTCACTCCCGTACTAACTGTCA

RSF1010 Backbone (continued); RSF1010 OriV Origin of Replication:
CGAACCCCTGCAATAACTGTCACGCCCCCCTGCAATAACTGTCACGAACCCCTGCAATAACTGTCACGCCCCCAAACCTGCAAACCCAGCAGGGGCGGGG
GCTGGCGGGGTGTTGGAAAAATCCATCCATGATTATCTAAGAATAATCCACTAGGCGCGGTTATCAGCGCCCTTGTGGGGCGCTGCTGCCCTTGCCCAAT
ATGCCCGGCCAGAGGCCGGATAGCTGGTCTATTCGCTGCGCTAGGCTACACACCGCCCCACCGCTGCGCGGCAGGGGGAAAGGCGGGCAAAGCCCGCT
AAACCCCACACCAAACCCCGCAGAAATACGCTGGAGCGCTTTTAGCCGCTTTAGCGGCCTTTCCCCCTACCCGAAGGGTGGGGGCGCGTGTGCAGCC

RSF1010 Backbone (continued): CCGCAGGGCCTGTCTCGGTCGATCAT

RSF1010 Backbone (continued); *Mobilisation Protein C* (antisense):
TCAGCCCGGCTCATCCTTCTGGCGTGGCGGCAGACCGAACAAGGCGCGGTCGTGGTCGCGTTCAAGGTACGCATCCATTGCCGCCATGAGCCGATCCTCC
GGCCACTCGCTGCTGTTCACCTTGGCCAAAATCATGGCCCCCACCAGCACCTTGCGCCTTGTTTCGTTCTTGCGCTCTTGCTGCTGTTCCCTTGCCCGCACCC
GCTGAATTTCGGCATTGATTCGCGCTCGTTGTTCTTCGAGCTTGGCCAGCCGATCCGCCGCCTTGTTGCTCCCCTTAACCAT

RSF1010 Backbone (continued):
CTTGACACCCCATTGTTAATGTGCTGTCTCGTAGGCTATCATGGAGGCACAGCGGCGGCAATCCCGACCCTACTTTGTAGGGGAGGGCGCACTTACCGGTT
TCTCTTCGAGAAACTGGCCTAACGGCCACCCTTCGGGCGGTGCGCTCTCCGAGGGCCATTGCATGGAGCCGAAAAGCAAAAGCAACAGCGAGGCAGC

RSF1010 Backbone (continued); *Mobilisation Protein A*:
ATGGCGATTTATCACCTTACGGCGAAAACCGGCAGCAGGTCGGGCGGCCAATCGGCCAGGGCCAAGGCCGACTACATCCAGCGCGAAGGCAAGTATGCC
CGCGACATGGATGAAGTCTTGCACGCCGAATCCGGGCACATGCCGGAGTTCGTCGAGCGGCCCGCCGACTACTGGGATGCTGCCGACCTGTATGAACGC
GCCAATGGGCGGCTGTTCAAGGAGGTCGAATTTGCCCTGCCGGTCGAGCTGACCCTCGACCAGCAGAAGGCGCTGGCGTCCGAGTTCGCCCAGCACCTG
ACCGGTGCCGAGCGCCTGCCGTATACGCTGGCCATCCATGCCGGTGGCGGCGAGAACCCGCACTGCCACCTGATGATCTCCGAGCGGATCAATGACGGC
ATCGAGCGGCCCGCCGCTCAGTGGTTCAAGCGGTACAACGGCAAGACCCCGGAGAAGGGCGGGGCACAGAAGACCGAAGCGCTCAAGCCCAAGGCATG
GCTTGAGCAGACCCGCGAGGCATGGGCCGACCATGCCAACCGGGCATTAGAGCGGGCTGGCCACGACGCCCGCATTGACCACAGAACACTTGAGGCGC
AGGGCATCGAGCGCCTGCCCGGTGTTCACCTGGGGCCGAACGTGGTGGAGATGGAAGGCCGGGGCATCCGCACCGACCGGGCAGACGTGGCCCTGAAC
ATCGACACCGCCAACGCCCAGATCATCGACTTACAGGAATACCGGGAGGCAATAGACC

RSF1010 Backbone (continued); *Mobilisation Protein A* (continued); *Mobilisation Protein B*:
ATGAACGCAATCGACAGAGTGAAGAAATCCAGAGGCATCAACGAGTTAGCGGAGCAGATCGAACCGCTGGCCCAGAGCATGGCGACACTGGCCGACGA
AGCCCGGCAGGTCATGAGCCAGACCCAGCAGGCCAGCGAGGCGCAGGCGGCGGAGTGGCTGAAAGCCCAGCGCCAGACAGGGGCGGCATGGGTGGA
GCTGGCCAAAGAGTTGCGGGAGGTAGCCGCCGAGGTGAGCAGCGCCGCGCAGAGCGCCCGGAGCGCGTCGCGGGGGTGGCACTGGAAGCTATGGCTA
ACCGTGATGCTGGCTTCCATGATGCCTACGGTGGTGCTGCTGATCGCATCGTTGCTCTTGCTCGACCTGACGCCACTGACAACCGAGGACGGCTCGATCTG
GCTGCGCTTGGTGGCCCGATGA

RSF1010 Backbone (continued); *Mobilisation Protein A* (continued); *Replication Protein B* (3 base overlap with *Mobilisation Protein B*):
ATGAAGAACGACAGGACTTTGCAGGCCATAGGCCGACAGCTCAAGGCCATGGGCTGTGAGCGCTTCGATATCGGCGTCAGGGACGCCACCACCGGCCAG
ATGATGAACCGGGAATGGTCAGCCGCCGAAGTGCTCCAGAACACGCCATGGCTCAAGCGGATGAATGCCCAGGGCAATGACGTGTATATCAGGCCCGCC
GAGCAGGAGCGGCATGGTCTGGTGCTGGTGGACGACCTCAGCGAGTTTGACCTGGATGACATGAAAGCCGAGGGCCGGGAGCCTGCCCTGGTAGTGGA
AACCAGCCCGAAGAACTATCAGGCATGGGTCAAGGTGGCCGACGCCGCAGGCGGTGAACTTCGGGGGCAGATTGCCCGGACGCTGGCCAGCGAGTACG
ACGCCGACCCGGCCAGCGCCGACAGCCGCCACTATGGCCGCTTGGCGGGCTTCACCAACCGCAAGGACAAGCACACCACCCGCGCCGGTTATCAGCCGT
GGGTGCTGCTGCGTGAATCCAAGGGCAAGACCGCCACCGCTGGCCCGGCGCTGGTGCAGCAGGCTGGCCAGCAGATCGAGCAGGCCCAGCGGCAGCAG
GAGAAGGCCCGCAGGCTGGCCAGCCTCGAACTGCCCGAGCGGCAGCTTAGCCGCCACCGGCGCACGGCGCTGGACGAGTACCGCAGCGAGATGGCCGG
GCTGGTCAAGCGCTTCGGTGATGACCTCAGCAAGTGCGACTTTATCGCCGCGCAGAAGCTGGCCAGCCGGGGCCGCAGTGCCGAGGAAATCGGCAAGG
CCATGGCCGAGGCCAGCCCAGCGCTGGCAGAGCGCAAGCCCGGCCACGAAGCGGATTACATCGAGCGCACCGTCAGCAAGGTCATGGGTCTGCCCAGC
GTCCAGCTTGCGCGGGCCGAGCTGGCACGGGCACCGGCACCCCGCCAGCGAGGCATGGACAGGGGCGGGCCAGATTTCAGCATGTAG

RSF1010 Backbone (continued):
TGCTTGCGTTGGTACTCACGCCTGTTATACTATGAGTACTCACGCACAGAAGGGGGTTTTATGGAATACGAAAAAAGCGCTTCAGGGTCGGTCTACCTGAT
CAAAAGTGACAAGGGCTATTGGTTGCCCGGTGGCTTTGGTTATACGTCAAACAAGGCCGAGGCTGGCCGCTTTTCAGTCGCTGATATGGCCAGCCTTAAC
CTTGACGGCTGCACCTTGTCCTTGTTCCGCGAAGACAAGCCTTTCGGCCCCGGCAAGTTTCTCGGTGACTGATATGAAAGACCAAAAGGACAAGCAGACC
GGCGACCTGCTGGCCAGCCCTGACGCTGTACGCCAAGCGCGATATGCCGAGCGCATGAAGGCCAAAGGGATGCGTCAGCGCAAGTTCTGGCTGACCGAC
GACGAATACGAGGCGCTGCGCGAGTGCCTGGAAGAACTCAGAGCGGCGCAGGGCGGGGGTAGTGACCCCGCCAGCGCCTAACCACCAACTGCCTGCAA
AGGAGGCAATCA

RSF1010 Backbone (continued); *Replication Protein A*:
ATGGCTACCCATAAGCCTATCAATATTCTGGAGGCGTTCGCAGCAGCGCCGCCACCGCTGGACTACGTTTTGCCCAACATGGTGGCCGGTACGGTCGGGG
CGCTGGTGTCGCCCGGTGGTGCCGGTAAATCCATGCTGGCCCTGCAACTGGCCGCACAGATTGCAGGCGGGCCGGATCTGCTGGAGGTGGGCGAACTGC
CCACCGGCCCGGTGATCTACCTGCCCGCCGAAGACCCGCCCACCGCCATTCATCACCGCCTGCACGCCCTTGGGGCGCACCTCAGCGCCGAGGAACGGCA
AGCCGTGGCTGACGGCCTGCTGATCCAGCCGCTGATCGGCAGCCTGCCCAACATCATGGCCCCGGAGTGGTTCGACGGCCTCAAGCGCGCCGCCGAGGG
CCGCCGCCTGATGGTGCTGGACACGCTGCGCCGGTTCCACATCGAGGAAGAAAACGCCAGCGGCCCCATGGCCCAGGTCATCGGTCGCATGGAGGCCAT
CGCCGCCGATACCGGGTGCTCTATCGTGTTCCTGCACCATGCCAGCAAGGGCGCGGCCATGATGGGCGCAGGCGACCAGCAGCAGGCCAGCCGGGGCA
GCTCGGTACTGGTCGATAACATCCGCTGGCAGTCCTACCTGTCGAGCATGACCAGCGCCGAGGCCGAGGAATGGGGTGTGGACGACGACCAGCGCCGGT
TCTTCGTCCGCTTCGGTGTGAGCAAGGCCAACTATGGCGCACCGTTCGCTGATCGGTGGTTCAGGCGGCATGACGGCGGGGTGCTCAAGCCCGCCGTGCT
GGAGAGGCAGCGCAAGAGCAAGGGGGTGCCCCGTGGTGAAGCCTAA

RSF1010 Backbone (continued); *Replication Protein C* (14 base overlap with *Replication Protein A*):
GTGGTGAAGCCTAAGAACAAGCACAGCCTCAGCCACGTCCGGCACGACCCGGCGCACTGTCTGGCCCCCGGCCTGTTCCGTGCCCTCAAGCGGGGCGAG
CGCAAGCGCAGCAAGCTGGACGTGACGTATGACTACGGCGACGGCAAGCGGATCGAGTTCAGCGGCCCGGAGCCGCTGGGCGCTGATGATCTGCGCAT
CCTGCAAGGGCTGGTGGCCATGGCTGGGCCTAATGGCCTAGTGCTTGGCCCGGAACCCAAGACCGAAGGCGGACGGCAGCTCCGGCTGTTCCTGGAACC
CAAGTGGGAGGCCGTCACCGCTGAATGCCATGTGGTCAAAGGTAGCTATCGGGCGCTGGCAAAGGAAATCGGGGCAGAGGTCGATAGTGGTGGGGCG
CTCAAGCACATACAGGACTGCATCGAGCGCCTTTGGAAGGTATCCATCATCGCCCAGAATGGCCGCAAGCGGCAGGGGTTTCGGCTGCTGTCGGAGTAC
GCCAGCGACGAGGCGGACGGGCGCCTGTACGTGGCCCTGAACCCCTTGATCGCGCAGGCCGTCATGGGTGGCGGCCAGCATGTGCGCATCAGCATGGA
CGAGGTGCGGGCGCTGGACAGCGAAACCGCCCGCCTGCTGCACCAGCGGCTGTGTGGCTGGATCGACCCCGGCAAAACCGGCAAGGCTTCCATAGATAC
CTTGTGCGGCTATGTCTGGCCGTCAGAGGCCAGTGGTTCGACCATGCGCAAGCGCCGCCAGCGGGTGCGCGAGGCGTTGCCGGAGCTGGTCGCGCTGG
GCTGGACGGTAACCGAGTTCGCGGCGGGCAAGTACGACATCACCCGGCCCAAGGCGGCAGGCTGA

RSF1010 Backbone (continued):
CCCCCCCCACTCTATTGTAAACAAGACATTTTTATCTTTTATATTCAATGGCTTATTTTCCTGCTAATTGGTAATACCATGAAAA
ATACCATGCTCAGAAAAGGC (Primer 149)
TTAACAATATTTTGAAAAATTGCCTACTGAGCGCTGCCGCACAGCTCCATAGGCCGCTTTCCTGGCTTTGCTTCCAGATGTATGCTCTTCTGCTCCTGC

Acknowledgements

I would like to thank Dr. Patrik Jones, my project supervisor, for all his support with this project and giving me the opportunity to work on research project in this interesting and unfamiliar area of molecular biology; as well as David Malatinszky, a finishing Ph.D. student who created the mutants and thus enabled this master's research project in the first place, to lead on from his own hard work. I am also grateful to Marine Valton, Ph.D. student in the microbial metabolic engineering group for all her help in guiding me through cyanobacterial techniques; and to Dr. John Rowland, Post-Doc in the MME group for his expert advice.