# In situ activity of NAC11-7 roseobacters in coastal waters off the Chesapeake Bay based on ftsZ expression.

Daohong Yao, Alison Buchan, Marcelino T Suzuki

6  ### *In situ* **Activity of NAC11-7 Roseobacters in Coastal Waters off the**

7  ### **Chesapeake Bay based on *ftsZ* Expression**

8  Daohong Yao[1], Alison Buchan[2] and Marcelino T. Suzuki[1,3*]

9  [1]Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science.
10  Solomons, MD, USA.
11  [2] Department of Microbiology, University of Tennessee Knoxville, Knoxville, TN, USA.
12

13  **\* to whom correspondence should be addressed:**

14  Université Pierre et Marie Curie (Paris VI) Laboratoire Arago and CNRS UMR 7621,

15  Laboratoire d'Océanographie Microbienne. Avenue du Fontaulé, Banyuls-sur-Mer 66650 France

16  **Electronic mail address:** suzuki@obs-banyuls.fr

17  **Tel:** +33(0)4 3019 2401 **FAX :** +33(0)4 6888 7398

18  **Running Title:** *In situ* expression of roseobacters *ftsZ*

19

20  _____

[3] Current Address: Université Pierre et Marie Curie (Paris 6) Laboratoire Arago and CNRS UMR 7621, Laboratoire d'Océanographie Microbienne, Banyuls-sur-Mer, France

## Summary

Determining *in situ* growth rates for specific bacterioplankton is of critical importance to understanding their contributions to energy and matter flow in the Ocean. Quantifying expression of genes central to cell division is a plausible approach for obtaining these measurements. In order to test this approach's assumptions, a quantitative PCR assay targeting the cell division gene *ftsZ* in the ubiquitous NAC11-7 group of the Rhodobacterales order of marine bacteria was developed. *ftsZ* genes and their corresponding mRNAs were measured in diel *in situ* samples and in parallel on-deck incubations. Strong correlations between *ftsZ* expression and gene abundance (R-squared=0.62) were observed *in situ*. Rapid changes in NAC11-7 *ftsZ* gene copies suggested that different populations from different water types were sampled with a significant positive correlation and between *ftsZ* expression and water temperature (R-squared=0.68, p<0.001). An outlier to this trend occurred at a single time point (9:00), which was remarkably consistent with a concomitant peak in *ftsZ* expression in on-deck incubations, suggesting the possibility of synchronous population growth.

# Introduction

Marine bacterioplankton play pivotal roles in oceanic food webs and the cycling of organic matter, which in turn influence global carbon pools (Azam and Malfatti, 2007; Wohlers et al., 2009). In recent years, the application of molecular, genomic, and transcriptomic techniques has provided us with vast datasets, greatly expanding our knowledge of bacterioplankton phylogenetic diversity, their environmental distributions, and metabolic capabilities (for reviews see Rappé and Giovannoni, 2003; Delong, 2009). Despite this knowledge, challenges still exist in understanding the ecological functions of **specific** bacterioplankton. Among these challenges is a lack of measurements of *in situ* growth rates of bacterioplankton at high phylogenetic resolution. This information is essential for elucidating the contributions of these ecotypes to biogeochemical cycles critical to the ecosystem.

The most commonly used approaches for determining growth rates of heterotrophic marine bacterioplankton are indirect and rely on measurements of bacterial production. Growth rates are often calculated from bacterioplankton production, which are usually estimated from the incorporation of $^{3}$H-thymidine or $^{3}$H-leucine (Fuhrman and Azam, 1982; Kirchman et al., 1985; Simon and Azam, 1989), or non-radioactive bromodeoxyuridine (Steward and Azam, 1999). These methods regard the entire bacterial community as a "black box". They provide community-level activity measurements, and therefore do not provide information on the production and growth rates of specific bacterioplankton. While the combination of microautoradiography with fluorescence *in situ* hybridization (i.e. Micro-FISH) allows the assessment of specific bacterioplankton activities at relatively narrow phylogenetic levels (i.e. Cottrell and Kirchman, 2003; Alonso and Pernthaler, 2005; Margolin, 2005), these experiments suffer from issues inherent to confinement and tracer-based studies (i.e. bottle effects).

57    A plausible approach to measure *in situ* activity, and perhaps growth rates of specific

58    bacterioplankton, involves quantifying the expression (mRNAs) of genes encoding proteins

59    involved in cell division.  The idea behind such approach would be to determine direct

60    relationships between *in situ* expression of protein-encoding genes and growth rates of specific

61    bacterioplankton, without additions or incubations.  The rationale is analogous to that of the

62    measurement of the frequency of dividing cells (Hagstrom et al., 1979), except that the

63    measurement could be made at specific phylogenetic levels, and earlier in the cell cycle (i.e.

64    before a clear septal ring is formed), thus decreasing problems associated with preferential

65    protistan grazing of dividing cells (Sherr et al., 1992).  Requirements for successfully using a

66    gene transcript tracking approach for **instantaneous** (i.e. at any time of the day) measurements

67    of growth would be that populations of targeted organisms do not divide synchronously, and that

68    the regulation of cell division protein synthesis occurs at the transcriptional level.  In cases where

69    the population divides synchronously, growth rates estimates are possible, but would not be

70    instantaneous and would require measurements throughout a diel cycle (i.e. Carpenter and Chang,

71    1988; Chang and Carpenter, 1988; Holtzendorff et al., 2002).

72    FtsZ initiates cell division of most prokaryotes by self-assembling into a membrane-

73    associated Z-ring structure and by recruiting other proteins to form the cell division septum

74    (Margolin, 2005; Dajkovic and Lutkenhaus, 2006; Osawa and Erickson, 2006). In synchronized

75    populations of *Caulobacter crescentus*, a model organism for the study of bacterial cell division,

76    the transcription of *ftsZ* is controlled by two regulators: DnaA promotes *ftsZ* transcription, while

77    CtrA its repressor, ensures that cell division is coordinated with DNA replication (Hottes et al.,

78    2005; McAdams and Shapiro, 2009). Levels of expression of *ftsZ* genes were also found to vary

79    during the cell cycle in synchronized *Prochlorococcus* populations in the Gulf of Aqaba, with

80  the maxima of expression at the replication phase (S phase; Holtzendorff et al., 2001;

81  Holtzendorff et al., 2002). These authors suggested that transcriptional regulation of *ftsZ* could

82  be a major factor triggering the observed synchronized cell division of *Prochlorococcus*

83  populations. Finally, the sequence of the FtsZ protein is highly conserved in most bacteria and its

84  phylogeny has good congruence to 16S rRNA phylogeny (Vaughan et al., 2004), allowing

85  putative identification of genes uncovered directly from the environment.

86      In this study, we investigated *in situ* expression of *ftsZ* genes putatively belonging to the

87  NAC11-7 subclade of the *Roseobacter* group of marine bacteria.  Members of the NAC11-7

88  clade have been implicated in DMSP degradation (Zubkov et al., 2001; Buchan et al., 2005) and

89  the genome sequence of a NAC11-7 representative (HTCC 2255) suggests members of this

90  subclade might be photoheterotrophic because it contains a proteorhodopsin coding gene

91  (Newton et al 2010). We quantified *ftsZ* expression of this group over a diel cycle to determine

92  whether evidence exists for synchronicity in cell division.  Gene expression was measured as the

93  ratio of *ftsZ* mRNA to *ftsZ* gene copies using quantitative real time PCR (qPCR) and reverse

94  transcription qPCR (RT-qPCR), respectively, in samples collected from a station off the

95  Chesapeake Bay and in a parallel on-board incubation.

96                              **Results**

97  ***Rhodobacterales ftsZ genes from coastal waters off the Chesapeake Bay.***

98      Degenerate primers targeting *ftsZ* from the order Rhodobacterales were designed based

99  on conserved regions identified in alignments of representative *ftsZ* sequences obtained from

100 GenBank.  A clone library was generated from fragments amplified from coastal Atlantic Ocean

101 surface waters and 74 *ftsZ* clones were analyzed.  Phylogenetic reconstruction of these sequenced

102 clones as well as representative reference sequences revealed three interesting groups (Figure 1):

103    a prevalent, yet unidentified group (designated Group I) containing 38 sequences  (51% of the

104    clones); a second unidentified group (Group II) with 31 sequences (42 % of the clones); and a

105    group represented by 5 sequences that were most closely related to the FtsZ sequence from strain

106    HTCC 2255 (Figure 1).  16S rRNA gene phylogeny places strain HTCC 2255 in the NAC11-7

107    group of the roseobacters (Buchan et al., 2005).   Given the previously reported global

108    distributions and abundances of NAC11-7 representatives and their putative photoheterotrophic

109    metabolism, this group was selected for further investigation.

110    ***In situ diel expression of NAC11-7 ftsZ genes.***

111           NAC11-7 *ftsZ* mRNA and DNA copies were monitored in surface waters over a 24 h

112    period at a single station located at the mouth of the Chesapeake Bay.  Reproducibility among

113    triplicates was quite good for gene copies (Figure 2), with a single exception of 21:00 where the

114    difference among samples was also reflected by different ARISA profiles (Supplemental Figure

115    1).  Estimated mRNA copies were more variable among triplicates, mostly likely due to RNA

116    degradation. In 5 of 9 timepoints, a single measurement was considered as an outlier, thus

117    averages of duplicates were taken. Gene copies, and more remarkably, gene expression, showed

118    rapid and sometimes large (c.a. 4-fold) changes with time (i.e. from 9:00 to 12:00 and from

119    18:00 to 21:00), suggesting distinct populations (i.e. patches) of NAC11-7 may have been

120    sampled at different times, as a result of water advection (Figure 2).  Interestingly, *ftsZ* gene

121    expression followed closely *ftsZ* gene abundance trends, except at the last two sampling times. A

122    regression analysis between *ftsZ* gene copies and gene expression using individual replicates

123    showed significant correlation ($R^2$= 0.6214, P=0.0003) for the first seven sampling times,

124    covering 18 hours from 6:00 to midnight. However, for the remaining two time points (3:00 and

125    6:00 on Sept 4, 2005) this trend no longer held. As a consequence, a correlation analysis of *ftsZ*

126    gene to gene expression inclusive of all sampling points, was not significant ($R^2$=0.0304,

127    P=0.45). These results indicate that at least for some of the samples a correlation existed between

128    gene abundance and per-cell gene expression activity.

129         In the first 12 hours, *ftsZ* expression and to some degree *ftsZ* gene copies appeared to be

130    somewhat correlated with the tidal cycle, with peaks of expression at 6:00 and 18:00 and lowest

131    expression at 12:00, lagging the onshore high and low tides by about 3 hours respectively (Figure

132    2).  Temperature and salinity also showed fluctuations that loosely followed the tidal cycle with

133    the same 3-hour lag (data not shown). After 18:00 a steady drop in temperature and simultaneous

134    increase in salinity was observed. Overall, these trends in salinity and temperature indicate that

135    tidal currents influenced our sampling site and that different waters with different NAC11-7

136    populations (or patches) were sampled.

137         In order to further examine the relationships between *ftsZ* gene abundance and expression

138    with water masses, we overlaid *ftsZ* gene abundances and expression to gene ratios onto

139    temperature-salinity (T-S) plots (Figure 3).  These plots indicate a cyclical variation between

140    warmer, less saline waters and cooler, more saline waters.  While, gene copies did not show clear

141    trends in the T-S plot (Supplemental Figure 2), *ftsZ* expression showed an interesting relationship.

142    With the single exception of the 9:00 sample, samples below 25.8°C showed significantly lower

143    *ftsZ* expression (two-sample t-test P=0.003) than those above 25.8°C (Figure 3).  A regression

144    analysis excluding the 9:00 measurements showed a significant correlation ($R^2$=0.68,

145    P=0.000025) between *ftsZ* expression and temperature (Figure 3).  These data strongly indicate

146    that populations from different water masses were sampled throughout the diel study.

147    Furthermore, an outlier in gene-normalized *ftsZ* transcript abundance is evident at the 9:00

148    sampling time, suggestive of a peak in *ftsZ* gene expression at that time (Figure 3).

149     *ARISA analysis of microbial communities.*

150          In order to examine the dynamics of bacterioplankton communities and populations at the

151     different sampling times, these communities were examined using automated ribosomal

152     intergenic spacer analysis (ARISA).  We observed that a number of ARISA peaks were present

153     at all time points analyzed, (i.e. 456, 485, 510 and 556 bp), while several peaks showed a more

154     ephemeral occurrence (i.e. 793, 799, 900 bp), suggesting that levels of patchiness differed for

155     different phylotypes (Supplemental Figure 1).  At least in the case of these fluctuating

156     phylotypes, different populations (patches) appear to have been sampled at different times.

157     Unfortunately, ARISA peaks with sizes corresponding to the NAC11-7 group were not readily

158     detected, as these organisms were likely present in numbers below the resolution of ARISA.

159     *Enclosed incubation diel expression of NAC11-7 ftsZ genes.*

160          A parallel on-deck incubation experiment in which *ftsZ* gene and gene expression were

161     quantified in incubated samples was conducted for comparative purposes. In these samples, *ftsZ*

162     gene abundance dropped from 6:00 to 15:00 and stabilized at ca.1 x $10^4$ copies/ml (a 2.5-fold

163     decrease).  Contrastingly, *ftsZ* expression showed a very remarkable 4-fold increase in

164     expression at 9:00 relative to the 6:00 time point.  However, by 12:00 expression was 5-fold less

165     (Figure 4).  Considering that the measurements were made in three separate, incubated bottles

166     and that the $C_T$s of the 9:00 cDNA samples were nearly two units lower than those of the

167     remaining samples, we are very confident that this observation was not due to an experimental

168     artifact.

169                                          **Discussion**

170          In order to test assumptions related to the measurement of *in situ* growth rates of specific

171     roseobacters based on cell division genes, a qPCR *ftsZ* assay was developed for the NAC11-7

172   clade.  Analysis of the NAC11-7 sequences reveals that although all the sequences were highly

173   similar at the amino acid level, one sequence, clone ftsZ05F3, was different at the DNA level

174   (Supplemental Figure 3).  Based on the ecotype theory (Cohan, 2001), clone ftsZ5F03 was

175   assumed to belong to a different ecotype that was not subjected to a theoretical selective sweep,

176   as were the remaining 5 phylotypes.  In order to maximize the chance that our measurements

177   reflected populations most coherent in their response to environmental factors, the sequence of

178   clone ftsZ05F03 was not targeted by our qPCR assay. This assay is highly specific to the

179   NAC11-7 ecotype represented by the 5 phylotypes described above, since specificity tests did

180   not show amplification for ftsZ05F03 nor any of 14 clones representing the unidentified clades

181   shown in Figure 1 (data not shown).

182       We observed that levels of gene-normalized transcripts were low. This was not

183   completely unexpected as mRNAs exist in a complex RNA pool composed primarily of rRNA

184   and have relatively short half-lives (Belasco, 1993). Thus, the efficiency of mRNA recovery is

185   expected to be considerably lower than that for genomic DNA.  While we did not measure

186   reverse transcription efficiencies here, we have previously shown RT efficiencies to be constant

187   between different samples for proteorhodopsin mRNAs using an analogous method with the

188   same reverse transcriptase and a specific primer (Shi, 2005).

189       It is important to emphasize that our measurements of gene expression are based on a *per*

190   *cell* average for the entire population. Thus, depending on how *ftsZ* is expressed in a single cell

191   during the cell cycle, the mRNA to gene ratio may reflect: 1) the fraction of the population that is

192   actively transcribing *ftsZ* in preparation for cell division or 2) the average per cell expression

193   level [i.e. if *ftsZ* is constitutively transcribed, and FtsZ regulation occurs post-transcriptionally in

194   preparation for cell division].  If a population displays synchronous growth and *ftsZ* expression

195    varies during the cell cycle (Case 1), we expect to observe peaks in the population-level *ftsZ*

196    expression. Conversely, if *ftsZ* transcription were constitutive (Case 2), one would not expect

197    *ftsZ* expression peaks even in synchronous populations.

198         The only other study to examine *ftsZ* gene expression in natural bacterial assemblages

199    was carried out with Red Sea populations of the cyanobacterium *Prochlorococcus*. In that study,

200    *ftsZ* gene expression was found to be highly synchronous, with a peak in expression (c.a. 10 fold

201    increase) occurring in the twilight hours before nightfall and lasting for over 2 hours

202    (Holtzendorff et al., 2002). These expression profiles were consistent with the known

203    photoautotrophic physiology of the organism; these populations are naturally synchronized and

204    undergo cell division at night (Vaulot and Partensky, 1992 ; Vaulot et al., 1995). *Caulobacter*

205    *crescentus*, an aquatic alphaproteobacterium, which is largely used as a model for cell cycle and

206    differentiation studies (see review by McAdams and Shapiro, 2009), is the closest roseobacter

207    relative for which extensive *ftsZ* gene expression studies have been undertaken. In this organism,

208    *ftsZ* gene expression is cell cycle-dependent and is time constrained (Quardokus et al., 1996;

209    Kelly et al., 1998; Sackett et al., 1998; Martin and Brun, 2000; Brun, 2001), with increases in

210    transcriptional activity greater than and 10-fold lasting for about 20% of the cell cycle (ca 30 min;

211    Kelly et al. 1998). Given the magnitude of the transcriptional response evident from these model

212    organisms and the expectation that natural bacterioplankton populations would have cell cycles

213    with lengths more similar to natural *Prochlorococcus* populations than *Caulobacter* laboratory

214    cultures, it is anticipated that a signature of elevated *ftsZ* gene expression would be evident in

215    natural NAC11-7 populations. Particularly, if these populations demonstrated some level of

216    synchronicity and the signal would not be masked by a portion of the population with basal level

217    expression. In contrast, FtsZ protein concentration per cell has been found to be constant

218    regardless of growth rate in the copiotrophic *E. coli (Gammaproteobacteria)* and *B. subtilis*

219    (*Firmicutes*) (Rueda et al., 2003; Weart and Levin, 2003; Haeusser and Levin, 2008). Several

220    arguments support time-constrained *ftsZ* expression in NAC11-7 members. As members of the

221    *Alphaproteobacteria*, these bacteria are more likely to share conserved features of central

222    physiology with *C. crescentus* than with the more distantly related *E. coli* or *B. subtilis*.  In

223    addition, the genome of NAC 11-7 isolate HTCC2255 contains the genes encoding for DnaA and

224    CtrA (blastp e-value <$10^{-20}$ using the *Caulobacter crescentus* amino acid sequences), the central

225    regulators of *ftsZ* expression and cell cycle in *C. crescentus* (McAdams and Shapiro, 2009).

226    Finally, it is difficult to rationalize advantages to NAC11-7, organisms adapted to somewhat

227    oligotrophic conditions, to synthesize a constant amount of FtsZ through the entire cell cycle

228    unless FtsZ has an alternative, unknown function.

229        Different lines of evidence indicate that different NAC11-7 populations (or patches) with

230    different growth rates were sampled at different time points during the *in situ* experiment. First,

231    remarkable fluctuations of NAC11-7 *ftsZ* gene copy numbers (nearly 4-fold in 3 h) were

232    observed *in situ* (Figure 2), implying changes in populations sizes resulting from physical

233    processes. Second, ARISA analysis of the total bacterioplankton community structure showed

234    evidence for patchiness, as certain phylotypes were only detected in specific time points

235    (Supplemental Figure 1). While transient populations were likely sampled throughout the diel

236    cycle, some trends in *ftsZ* gene and gene expression did emerge that revealed insight into *ftsZ*

237    gene expression profiles and synchronicity of these populations. During the first seven data

238    points (18 hrs from 6:00 to 0:00), a strong correlation ($R^2$= 0.6214) was observed between

239    NAC11-7 abundance *in situ* (inferred from *ftsZ* copies/ml), and the gene-normalized *ftsZ*

240    transcript abundance, indicating that levels of expression are not constant in this ecotype. This

241    was suggestive that more active populations yielded, or were associated, with higher abundances

242    of these organisms. Collectively, these results support the case for non-synchronous cell division

243    since *ftsZ* expression was not constrained to specific times (i.e. *ftsZ* was expressed at all

244    measured time points). Finally, the plot of gene-normalized expression over a temperature-

245    salinity diagram, and a regression analysis showed that, with the exception of the 9:00 replicates,

246    there is a high correlation between water temperature and *ftsZ* expression (Figure 3).

247    We found a remarkable peak of gene-normalized *ftsZ* transcript abundance at 9:00 in the

248    on-deck incubation experiment, which could be interpreted in several ways. First, and in

249    contrast to conclusions drawn from the *in situ* samples, *ftsZ* expression (and by extension cell

250    division) could be synchronous in the putatively photoheterotrophic NAC11-7 clade. An

251    alternative explanation would be that the 9:00 peak of expression resulted from stimulation

252    caused by confinement. The observations that 1) the response was fast and time-constrained; 2)

253    gene abundances steadily decreased with time, and 3) there is no evidence that members of the

254    NAC11-7 group respond positively to enrichment conditions (i.e. this group is not known as

255    copiotrophic), all argue against this notion. The slow decline in *ftsZ* gene abundance in the

256    bottles might be explained by a lack of growth, and continuous mortality by viral lysis and/or

257    protistan bacterivory. The peak of *ftsZ* expression at 9:00 could be explained by a previous

258    "commitment" of the circadian rhythm. As different NAC11-7 populations (patches) were

259    sampled over the course of the *in situ* diel it is difficult to directly compare *in situ ftsZ* gene

260    expression levels with the parallel on-deck experiment. However, the fact that the *in situ* sample

261    from 9:00 was higher than expected based on the correlation between gene expression and

262    temperature (Figure 3) corroborates the incubation results, although we only sampled a single

263    diel cycle and there is a possibility that this was a fortuitous coincidence.

264        An intriguing interpretation of these seemingly contradictory results is that a certain

265        fraction of the population could be synchronously dividing, with a peak in *ftsZ* expression at 9:00,

266        while a background of asynchronously dividing cells could also exist, some of which would be

267        expressing *ftsZ* at any given time. This "background" expression would be in fact, better

268        correlated to temperature or other parameters influencing growth. The fact that a certain level of

269        *ftsZ* expression was measured throughout the diel cycle in synchronously dividing

270        *Prochlorococcus* populations (Holzendorf et al 2001) supports this model.  However, in the case

271        of *Prochlorococcus*, *ftsZ* expression is at the end of the light period and cell division occurs at

272        the dark (Holtzendorff et al., 2001).

273        To our knowledge, this is the first study measuring the expression of cell division genes

274        by specific heterotrophic bacterioplankton and to show that *ftsZ* expression varies between

275        different populations of a heterotrophic ecotype. The possibility of synchronous growth while

276        intriguing will need further exploration using combined incubation and *in situ* measurements.

277        However the latter should include Lagrangian sampling to minimize advective effects, and allow

278        measurement in coherent populations.

279

280                      **Experimental Procedures**

281        ***Sampling***

282        Surface water samples were collected every 3 hours from 6:00 local time on September 3

283        to 6:00 on September 4, 2005 at station RM6 (37°05.61N, 75°42.35 W) aboard the RV Cape

284        Henlopen.  Temperature and salinity were measured using a SBE9 CTD (Seabird, Bellevue,

285        WA), and tidal effects were estimated from the height of the tide at Ship Shoal Inlet calculated

286        using the data at the NOAA tides and current prediction page http://tidesandcurrents.noaa.gov.

287 Triplicate 590 ml water subsamples were collected and pre-filtered through GF/A filters (1.6 $\mu$m

288 nominal pore; Whatman, Maidstone, UK). Ninety ml of pre-filtered sample was filtered through

289 13 mm diameter 0.2$\mu$m Supor200® polysulfone filters (Pall Corp., East Hills, NY) and the filters

290 were transferred to a tube containing 130µl lysis buffer (2 mM NaEDTA (pH 8.0), 20 mM

291 Tris•Cl (pH 8.0), 1.2% v/v Triton X100). The remaining 500 ml were filtered through 25 mm

292 diameter 0.2 $\mu$m Supor200® filters (Pall Gelman Inc.) and transferred to screw cap tubes

293 containing 250 µl RNALater (Ambion, Austin, TX). All samples were frozen at –20ºC aboard

294 and within a week stored at –70ºC until nucleic acid extraction.

295     On-deck incubation experiments were conducted using water collected at the first

296 sampling time (6:00, 03 September, 2005). Fifteen l of surface water was used to fill 24 x 500

297 ml (600 ml total volume) polycarbonate bottles (Nalge Nunc International Corp., Rochester, NY),

298 and incubated at *in situ* temperatures in an on-deck incubator. At every *in situ* sampling point,

299 three of the incubation bottles were taken and sampled for nucleic acids as described for cast

300 samples.

301 ***Nucleic acid extraction and quantification***

302     Total DNA was extracted from Supor200® 0.2 $\mu$m filters as previously described, except

303 that the DNeasy 96 Tissue Kit was used, and therefore the DNeasy 96 plates were sealed and

304 centrifugations performed in a Sorvall® Legend™ T Centrifuge with a Highplate® rotor (Kendro,

305 Osterode, Germany) at 5250 rpm for 10 min for binding and 5250 rpm for 5 min for washes. The

306 DNeasy 96 plate was incubated at 70ºC for 15 min evaporate traces of ethanol and DNA was

307 eluted by centrifugation at 5250 rpm, for 2 min.

308     Total RNA was extracted from Supor200® 0.2 $\mu$m filters following an optimized protocol

309 adapted from the Qiagen RNeasy® 96 manual (Shi 2005). Filters in screw cap tubes were thawed

310     on the ice, along with the RNAlater, which was not removed prior to RNA extraction. Low

311     protein binding zirconium oxide beads (equivalent to ca 320 $\mu$l) were added into each tube (200

312     $\mu$m, OPS Diagnostics, Lebanon, NJ). ß-Mercaptoethanol was mixed in a 1:100 ratio into RLT

313     buffer and 875 $\mu$l of the mixture added to screw cap tubes. The tubes were beaten in a MM301

314     mixer mill (Retsch GmbH Inc., Haan, Germany) at 30.0 HZ for 2 min and incubated for 5 min at

315     70 ºC. 800 $\mu$l of the liquid phase was transferred into a new low-RNA-binding 2 ml

316     microcentrifuge tube (Ambion, Inc.), avoiding the beads. 800 $\mu$l of 100% ethanol was added into

317     each tube and well mixed well. 800 $\mu$l of the mixture was loaded into wells of RNeasy 96 plate,

318     sealed and centrifuged at 5000 rcf for 5 min, and the procedure repeated. The columns were

319     washed once with 800 $\mu$l RW1 buffer and twice with 800 $\mu$l RPE buffer at 5000 rcf for 5 min,

320     with the last spin for 15 min. 35 $\mu$l Diethylpyrocarbonate (DEPC) treated water (Ambion) were

321     added into each column, incubation for 1 min at room temperature and 5 min centrifugation at

322     5000 rcf.  This procedure was repeated with another 35 $\mu$l DEPC treated water. RNA was treated

323     the DNA-free™ kit (Ambion) to remove co-extracted DNA.

324         DNA and RNA concentrations were quantified fluorometrically by PicoGreen® and

325     RiboGreen® staining respectively (Molecular Probes, Invitrogen Corp., Carlsbad, CA) on a

326     Spectra MAX Gemini microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA).

327     ***Construction of ftsZ DNA PCR clone library***

328         An initial *ftsZ* and FtsZ database was developed using *arb* (Ludwig *et al*., 2004) by

329     importing gene sequences listed by Vaughan and coworkers (Vaughan et al., 2004). 163

330     representative FtsZ sequences were exported and used to retrieve additional aminoacid, and

331     coding gene sequences using blastp searches against the March 2005 NCBI *nt, env_nt* and *wgs*

332     databases. All aminoacid sequences were aligned using ClustalW (Thompson et al., 1994) and an

15

333     bayesian tree containing 507 sequences (144 homologous positions) was constructed using

334     MrBayes version 3.0 (Ronquist and Huelsenbeck, 2003) with the following parameters: 800,000

335     generations, mixed models of amino acid substitution, and a burn in of 6000 trees.  In this tree,

336     the *Alphaproteobacteria* formed a monophyletic clade with high confidence values and several

337     orders including *Rhizobiales* and *Rhodobacterales*, which also formed monophyletic clades (data

338     not shown). The database was updated with public sequences belonging to the *Rhodobacterales*,

339     and was used for the design of PCR primers and probes.  Degenerate primers targeting *ftsZ* from

340     *Rhodobacterales* (ftsZrb2F: 5'-AAY GCN GTS AAY AAY AT-3', ftsZrb2R: 5'-YTT NCC

341     CAT YTC RT-3') were designed and used to retrieve *ftsZ* gene sequences by PCR and cloning

342     from a DNA sample collected at time zero.

343          1 $\mu$l of extracted genomic DNA was used as template in a 10 $\mu$l-volume PCR reaction,

344     which also included 10X PCR buffer, 0.2 mM of each dNTP, 3 mM $MgCl_2$, 500 nM forward

345     primer ftsZ2rbF, 500 nM reverse primer ftsZ2rbR and 0.025 U/$\mu$l of Platinum® Taq DNA

346     Polymerase (Invitrogen, Carlsbad, CA). Reactions were performed on a GeneAmp 9700 PCR

347     system (Applied Biosystems) and cycling conditions were as follows: 2 min at 94 °C and 37

348     cycles of 30 sec at 94 °C, 30 sec at 55 °C and 2 min at 72 °C. PCR was followed by a

349     reconditioning (Thompson et al., 2002) of 5 steps. Reconditioned PCR products were loaded on

350     a 1% modified TAE (40 mM Tris-acetate, pH 8.0, 0.1 mM $Na_2EDTA$) agarose gel and separated

351     by electrophoresis. Target fragments were cut, recovered by Ultrafree®-DA (Millipore) gel

352     extraction and used to built a *ftsZ* gene clone library using the TOPO™ TA cloning kit

353     (Invitrogen) following manufacture's instructions. 96 clones were bidirectionally sequenced

354     using BigDye V3.1 chemistry and capillary electrophoresis on an AB3100 genetic analyzer

355     (Applied Biosystems Inc, Foster City, CA). These sequence data have been submitted to the

356    GenBank database under accession number HM035149-HM035222.  74 sequences identified as

357    *ftsZ* were imported into the ARB database described above and added to the tree described above

358    using the ADD_BY_PARSIMONY tool.  Finally, a bayesian tree of Rhodobacterales *ftsZ* was

359    constructed using the MPI version of Mr Bayes V3.1 (Ronquist and Huelsenbeck, 2003; Altekar

360    et al., 2004). 188 homologous amino acid positions from 123 sequences were exported and used

361    in this phylogenetic analysis. Two four parallel chains of 2,000,000 generations were run with

362    mixed models of amino acid substitution; trees were sampled every 100 generations, and 11000

363    "burnin" trees were excluded to generate the consensus tree.  The average standard deviation of

364    split frequencies was below 0.05 after 1,100,000 generations.

365    ***Real Time PCR Assays***

366         PCR primers and a Taqman probe were designed to target selected members of the

367    NAC11-7 group.  Results of *ftsZ* cloning and sequencing indicated that based on amino acid

368    sequences, five clone sequences were affiliated with strain HTCC 2255. However, the DNA

369    sequence of one of these clones (ftsZ5F03) contained obviously higher variation to the remainder

370    clones and HTCC 2255 (Supplemental Figure 3). Based on ecotype theory (Cohan, 2001), it

371    appeared that the distinct clone could belong to a different ecotype, which we assumed was not

372    subject to a periodic selection event as the remaining sequences. Thus we designed a primer and

373    probe to exclusively target the remaining four sequences and HTCC 2255. The primer and the

374    probe were manually designed with aid of probe match functions in the arb_edit module of the

375    ARB package. $T_M$, secondary structure and possible dimers were checked using Primer Express

376    (Applied Biosystems) and the Oligo Analyzer online tool (www.idtdna.com).

377         To test specificity of primers to the target sequences, 3 target clones and 15 non-target

378    clones were purified using QuickLyse Miniprep kit (Qiagen), and diluted to $10^7$ *ftsZ* copies/$\mu$l

379     with nuclease-free TE buffer (Ambion). Ten $\mu$l PCR reactions contained 5 $\mu$l TaqMan®

380     Universal PCR Master Mix (Applied Biosystems), 0.5$\mu$M Forward primer ftsZrbA03-2qF (GTG

381     AAA AAG CTA CTG AGG GTC T) and Reverse primer ftsZrbA03-2qR (GCT TCC TGC CAG

382     ATG ATC), and 1 $\mu$l plasmid template. The cycling parameters were as follows:  2 min at 50 °C,

383     10 min at 95 °C and 30 cycles of 15 sec at 95 °C and 1min at 57 °C. All PCR products were

384     loaded in a 2% NuSieve® (3:1) Agarose Gel (Cambrex, Rockland, ME), electrophoresed, and

385     post-stained with 1:10,000 SYBR® Gold (Invitrogen) for 30 min.  The gel was visualized with a

386     FluoroChem 8900 (Alpha Innotech, San Leandro, CA).


387        In order to get the highest amplification efficiency in real time PCR, a primer

388     concentration matrix was performed.  In each 25 $\mu$l reaction, the following reagents were kept

389     constant: 1X PCR buffer, 0.2 mM of dATP, dGTP and dCTP, 0.4 mM of dUTP, 5 mM $MgCl_2$,

390     200 nM probe NAC11-7 (AAC CAACAGTAGGAGCATTAGCCGCT), 1.2 $\mu$M SuperROX™

391     (Biosearch Technologies, Novato, CA), 0.01 U/$\mu$l AmpErase® Uracil N-glycosylase (UNG)

392     (Applied Biosystems), 0.025 U/$\mu$l of Platinum® Taq DNA Polymerase (Invitrogen), 2.5 $\mu$l

393     NAC11-7 standard ($10^4$ copies/$\mu$l) and a matrix of forward and reverse primer concentrations of

394     0.1 $\mu$M, 0.5 $\mu$M, 1.0 $\mu$M, 1.5 $\mu$M. Reactions were set in a MicroAmp® Optical 96-Well

395     Reaction Plate (Applied Biosystems), which was sealed with an optical adhesive cover (Applied

396     Biosystems) reactions and ran in an ABI Prism 7000 Sequence Detection system, following the

397     cycling parameters: 2 min at 50 °C, 10 min at 95 °C and 40 cycles of 15 sec at 95 °C and 1 min

398     at 57 °C.  The primer combination yielding the lowest $C_T$ (1.5 $\mu$M ftsZrbA03-2qF; 0.5 $\mu$M

399     ftsZrbA03-2qR) was used in all subsequent measurements.

400        A plasmid containing cloned DNA that was purified and linearized as previously

401     described (Suzuki et al., 2000) was used to prepare standards for real time PCR for quantification

402 of *ftsZ* gene and mRNA. 2.5 $\mu$l of DNA extracts were used in 25 $\mu$l reactions and standards

403 ranged from $10^2$ to $10^7$ copies/$\mu$l. Reactions were run in duplicate along with non-template

404 controls using the same cycling parameters described for the primer matrix. *ftsZ* cDNA was

405 quantified in the same manner as *ftsZ* gene except that 5 $\mu$l of Reverse transcription products

406 were used as template. All real time PCR measurements were calculated as copy numbers per

407 volume of seawater, assuming that nucleic acid extraction efficiencies were constant as shown by

408 Shi (Shi, 2005). mRNAs copies were assumed equal to cDNA copies. Since DNA and mRNA in

409 the *in situ* experiment were measured in triplicate biological samples, *ftsZ* genes and mRNA

410 copy numbers that were >2X or <X/2 (X is the average of the remainder two replicates) were

411 treated as outliers and removed from the analyses. Incubated samples were not subjected to

412 outlier analysis, but the DNA samples at 3:00 were lost and not included in the analysis.

413 **Optimization of Reverse Transcription**

414 Since preliminary results showed relatively high $C_T$ values from *in situ* sample cDNAs,

415 we attempted to increase *ftsZ* cDNA copy numbers by: (1). Increasing reverse transcription

416 efficiency, by varying template amount, RT temperature and RT duration and (2). Adding

417 increased amounts of cDNA products in real-time PCR reactions. In optimized conditions 4 $\mu$l

418 purified RNA (ca. 10 ng) was reverse transcribed to cDNA using ThermoScript™ Reverse

419 Transcriptase. Template and 10 pmol of the NAC11-7 specific primer ftsZrbA03-2qR were

420 heated to 65°C for 5 min and chilled on ice immediately, followed by the addition of 5X cDNA

421 synthesis buffer, 5mM DTT, 2 U/$\mu$l RNaseOUT™ Inhibitor (Invitrogen), 0.75 U/$\mu$ of reverse

422 transcriptase and DEPC-treated water to a final volume of 10 $\mu$l. Reactions were incubated at

423 55°C for 60 min to synthesize cDNA and at 85°C for 5 min to inactivate the transcriptase. No-

424    RT controls were performed for one of the triplicate samples using the same method except

425    substituting reverse transcriptase with DEPC-treated water.

426    ***ARISA (Automated rRNA intergenic spacer analysis)***

427    One $\mu$l environmental genomic DNA was used to perform a 10 $\mu$l ARISA reactions,

428    containing 1X PCR buffer, 1.2 mM MgCl$_2$, 0.08 mM dNTPs (Promega Corp., Madison, WI), 0.5

429    $\mu$M primer 1406F-FAM (Fisher and Triplett, 1999), 1.5 $\mu$M primer 23S-Y (Dyda et al., 2009),

430    and 0.01 U/$\mu$l of Platinum® Taq DNA Polymerase (Invitrogen). Reactions were run on a

431    GeneAmp 9700 (Applied Biosystems) under the following conditions:  Initial denaturation and

432    enzyme activation at 94 ºC for 2 min, followed by 35 cycles of 94 ºC for 30 sec, 55 ºC for 30 sec

433    and 65 ºC for 2min. 1 $\mu$l of each PCR reaction was mixed with 9 $\mu$l of 1:0.06 Formamide and

434    GS2500 size standard (Applied Biosystems), denatured at 94 ºC for 2 min and separated by

435    capillary electrophoresis using an Applied Biosystems 3100 genetic analyzer. Sizes of the

436    fragments were analyzed by the Peak Scanner™ Software v1.0 (Applied Biosystems).

437    **Acknowledgements**

441

## References

441  Alonso, C., and Pernthaler, J. (2005) Incorporation of glucose under anoxic conditions by

442  bacterioplankton from coastal North Sea surface waters. *Appl Environ Microbiol* **71**: 1709-

443  1716.

444  Altekar, G., Dwarkadas, S., Huelsenbeck, J.P., and Ronquist, F. (2004) Parallel Metropolis

445  coupled Markov chain Monte Carlo for Bayesian phylogenetic inference. *Bioinformatics* **20**:

446  407-415.

447  Azam, F., and Malfatti, F. (2007) Microbial structuring of marine ecosystems. *Nat Rev Microbiol*

448  **5**: 782-791.

449  Belasco, J.G. (1993) mRNA degradation in prokaryotic cells: an overview. . In *Control of*

450  *Messenger RNA Stability*. Belasco, J.G., Brawerman, G. (ed). San Diego CA: Academic

451  Press, pp. 3-11.

452  Brun, Y.V. (2001) Global analysis of a bacterial cell cycle: tracking down necessary functions

453  and their regulators. *Trends Microbiol* **9**: 405-407.

454  Buchan, A., Gonzalez, J.M., and Moran, M.A. (2005) Overview of the marine *Roseobacter*

455  lineage. *Appl Environ Microbiol* **71**: 5665-5677.

456  Carpenter, E.J., and Chang, J. (1988) Species-Specific Phytoplankton Growth-Rates Via Diel

457  DNA-Synthesis Cycles .1. Concept of the Method. *Mar Ecol-Prog Ser* **43**: 105-111.

458  Chang, J., and Carpenter, E.J. (1988) Species-Specific Phytoplankton Growth-Rates Via Diel

459  DNA-Synthesis Cycles .2. DNA Quantification and Model Verification in the

460  Dinoflagellate Heterocapsa-Triquetra. *Mar Ecol-Progr Ser* **44**: 287-296.

461  Cohan, F.M. (2001) Bacterial species and speciation. *Syst Biol* **50**: 513-524.

463    Cottrell, M.T., and Kirchman, D.L. (2003) Contribution of major bacterial groups to bacterial

464        biomass production (thymidine and leucine incorporation) in the Delaware estuary. *Limnol*

465        *Oceanogr* **48**: 168-178.

466    Dajkovic, A., and Lutkenhaus, J. (2006) Z ring as executor of bacterial cell division. . *J Mol*

467        *Microbiol Biotechnol* **11**: 140-151.

468    Delong, E.F. (2009) The microbial ocean from genomes to biomes. *Nature* **459**: 200-206.

469    Dyda, R.Y., Suzuki, M.T., Yoshinaga, M.Y., and Harvey, H.R. (2009) The response of microbial

470        communities to diverse organic matter sources in the Arctic Ocean. *Deep-Sea Research*

471        *Part Ii-Topical Studies in Oceanography* **56**: 1249-1263.

472    Fisher, M.M., and Triplett, E.W. (1999) Automated Approach for Ribosomal Intergenic Spacer

473        Analysis of Microbial Diversity and Its Application to Freshwater Bacterial Communities.

474        *Appl Environ Microbiol* **65**: 4630-4636.

475    Fuhrman, J.A., and Azam, F. (1982) Thymidine incorporation as a measure of heterotrophic

476        bacterioplankton production in marine surface waters: evaluation and field results *Mar Biol*

477        **66**: 109-120.

478    Haeusser, D.P., and Levin, P.A. (2008) The great divide: coordinating cell cycle events during

479        bacterial growth and division. *Curr Opin Microbiol* **11**: 94-99.

480    Hagstrom, A., Larsson, U., Horstedt, P., and Normark, S. (1979) Frequency of dividing cells, a

481        new approach to the determination of bacterial growth rates in aquatic environments. *Appl*

482        *Environ Microbiol* **37**: 805-812.

483    Holtzendorff, J., Marie, D., Post, A.F., Partensky, F., Rivlin, A., and Hess, W.R. (2002)

484        Synchronized expression of ftsZ in natural Prochlorococcus populations of the Red Sea.

485        *Environ Microbiol* **4**: 644-653.

486    Holtzendorff, J., Partensky, F., Jacquet, S., Bruyant, F., Marie, D., Garczarek, L. et al. (2001)

487         Diel Expression of Cell Cycle-Related Genes in Synchronized Cultures of Prochlorococcus

488         sp. Strain PCC 9511. *J Bacteriol* **183**: 915-920.

489    Hottes, A.K., Shapiro, L., and McAdams, H.H. (2005) DnaA coordinates replication initiation

490         and cell cycle transcription in Caulobacter crescentus. *Mol Microbiol* **58**: 1340-1353.

491    Kelly, A.J., Sackett, M.J., Din, N., Quardokus, E., and Brun, Y.V. (1998) Cell cycle-dependent

492         transcriptional and proteolytic regulation of FtsZ in Caulobacter. *Genes Dev* **12**: 880-893.

493    Kirchman, D.L., K'Nees, E., and Hodson, R. (1985) Leucine incorporation and its potential as a

494         measure of protein synthesis by bacteria in natural aquatic systems. *Appl Environ*

495         *Microbiol* **49**: 599-607.

496    Ludwig W., Strunk O., Westram R., Richter L., Meier H., Yadhukumar, et al. (2004) ARB: a

497         software environment for sequence data. Nucleic Acids Res 32: 1363-1371

498    McAdams, H.H., and Shapiro, L. (2009) System-level design of bacterial cell cycle control.

499         *FEBS Lett* **583**: 3984-3991.

500    Margolin, W. (2005) FtsZ and the division of prokaryotic cells and organelles. *Nat Rev Mol Cell*

501         *Biol* **6**: 862-871.

502    Martin, M.E., and Brun, Y.V. (2000) Coordinating development with the cell cycle in

503         Caulobacter. *Curr Opin Microbiol* **3**: 589-595.

504    Newton, R.J., Griffin, L.E., Bowles, K.M., Meile, C., Gifford, S., Givens, C.E., Howard, E.C.,

505         King, E., Oakley, C.A., Reisch, C.R., Rinta-Kanto, J.M., Sharma, S., Sun, S., Varaljay, V.,

506         Vila-Costa, M., Westrich, J.R., Moran, M.A. (2010) Genome characteristics of a generalist

507         marine bacterial lineage. ISME J **4**:784-798.

508 Osawa, M., and Erickson, H.P. (2006) FtsZ from divergent foreign bacteria can function for cell

509    division in Escherichia coli. *J Bacteriol* **188**: 7132-7140.

510 Quardokus, E.M., Din, N., and Brun, Y.V. (1996) Cell cycle regulation and cell type-specific

511    localization of the FtsZ division initiation protein in Caulobacter. *Proc Natl Acad Sci* **93**:

512    6314-6319.

513 Rappé, M.S., and Giovannoni, S.J. (2003) The uncultured microbial majority. *Annu Rev*

514    *Microbiol* **57**: 369-394.

515 Ronquist, F., and Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under

516    mixed models. *Bioinformatics* **19**: 1572-1574.

517 Rueda, S., Vicente, M., and Mingorance, J. (2003) Concentration and assembly of the division

518    ring proteins FtsZ, FtsA, and ZipA during the Escherichia coli cell cycle. *J Bacteriol* **185**:

519    3344-3351.

520 Sackett, M.J., Kelly, A.J., and Brun, Y.V. (1998) Ordered expression of ftsQA and ftsZ during

521    the Caulobacter crescentus cell cycle. *Mol Microbiol* **28**: 421-434.

522 Sherr, B.F., Sherr, E.B., and McDaniel, J. (1992) Effect of protistan grazing on the frequency of

523    dividing cells in bacterioplankton assemblages. *Appl Environ Microbiol* **58**: 2381-2385.

524 Shi, Y. (2005) Measurement of in situ expression of proteorhodopsin genes at the North Pacific

525    Central Gyre Station ALOHA. MSc. Thesis, University of Maryland College Park, p. 127.

526 Simon, M., and Azam, F. (1989) Protein content and protein synthesis rates of planktonic marine

527    bacteria. *Mar Ecol-Prog Ser* **51**: 201-213.

528 Steward, G.F., and Azam, F. (1999) Bromodeoxyuridine as an alternative to H-3-thymidine for

529    measuring bacterial productivity in aquatic samples. *Aquat Microb Ecol* **19**: 57-66.

530    Suzuki, M.T., Taylor, L.T., and Delong, E.F. (2000) Quantitative analysis of small-subunit

531        rRNA genes in mixed microbial populations via 5'-nuclease assays. *Appl Environ*

532        *Microbiol* **66**: 4605-4614.

533    Thompson, J.D., Higgins, D.G., and Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity

534        of progressive multiple sequence alignment through sequence weighting, position-specific

535        gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673-4680.

536    Thompson, J.R., Marcelino, L.A., and Polz, M.F. (2002) Heteroduplexes in mixed-template

537        amplifications: formation, consequence and elimination by 'reconditioning PCR'. *Nucleic*

538        *Acids Res* **30**: 2083-2088.

539    Vaughan, S., Wickstead, B., Gull, K., and Addinall, S.G. (2004) Molecular evolution of FtsZ

540        protein sequences encoded within the genomes of archaea, bacteria, and eukaryota. *J Mol*

541        *Evol* **58**: 19-29.

542    Vaulot, D., and Partensky, F. (1992 ) Cell Cycle Distributions of Prochlorophytes in the North

543        Western Mediterranean Sea  *Deep Sea Res*  **39(5)**: 727-742 511

544    Vaulot, D., Marie, D., Olson, R.J., and Chisholm, S.W. (1995) Growth of Prochlorococcus, a

545        Photosynthetic Prokaryote, in the Equatorial Pacific-Ocean. *Science* **268**: 1480-1482.

546    Weart, R.B., and Levin, P.A. (2003) Growth rate-dependent regulation of medial FtsZ ring

547        formation. *J Bacteriol* **185**: 2826-2834.

548    Wohlers, J., Engel, A., Zollner, E., Breithaupt, P., Jurgens, K., Hoppe, H.G. et al. (2009)

549        Changes in biogenic carbon flow in response to sea surface warming. *Proc Natl Acad Sci*

550        **106**: 7067-7072.

551    Zubkov, M.V., Fuchs, B.M., Archer, S.D., Kiene, R.P., Amann, R., and Burkill, P.H. (2001)

552        Linking the composition of bacterioplankton to rapid turnover of dissolved

553    dimethylsulphoniopropionate in an algal bloom in the North Sea. *Environ Microbiol* **3**:

554    304-311.


555
556


557                                        **Figure legends**

558    Figure 1.  Consensus bayesian phylogenetic tree of FtsZ protein sequences from the

559    *Rhodobacterales* group reconstructed using MrBayes v3.1. FtsZ sequences retrieved in this study

560    are marked in boldface. The numbers on nodes represent branch confidence values. The bar

561    represents the mean number of substitutions per residue in the trees used to build the consensus

562    tree. Following clone or group designations, GenBank accession numbers are provided.


563    Figure 2. *FtsZ* gene copy numbers (grey bars) and expression (estimated from the ratio of *ftsZ*

564    cDNA copies to gene copies; white bars) at station RM6 on September 3 and 4, 2005, and tidal

565    heights at Ship Shoal Inlet (37° 13′N, 75° 48′W). Error bars represent the standard deviation of

566    triplicate samples from a single CTD cast. A "*" represent samples where average of two bottles

567    were calculated; "**" represents a sample where standard deviation was estimated from

568    duplicate DNA and mRNA measurements while the ratio was calculated from a single bottle

569    where both DNA and mRNA measurements existed. Pre-dawn and post-dusk sampling times are

570    represented by the grayed areas.  The tidal height is shown with the line lacking symbols.


571    Figure 3. Temperature and Salinity plot, with overlaying *in situ* NAC11-7 *ftsZ* expression.  Gene

572    normalized *ftsZ* expression levels are proportional to the area of the circles.  The area of the

573    circle representing the 3:00 PM sample corresponds to $2.5 \times 10^{-4}$ mRNA copies per gene copies.

574    Inset: Linear regression of gene-normalized *ftsZ* transcripts and temperature excluding the 9:00

575    data points which were overlaid onto the same plot (open squares within the oval); R-

576    squared=0.68, p<0.001.

577    Figure 4. *ftsZ* gene copy numbers (filled squares) and expression (estimated from the ratio of *ftsZ*

578    cDNA copies to gene copies; open circles) in samples incubated onboard. Error bars represent

579    the standard deviation of triplicate bottles.

580    Supplemental Figure 1. Percentage of different phylotypes identified by ARISA fragment sizes

581    in the *in situ* samples. The results shown in here indicate that the replicate subsamples were

582    largely similar in composition, indicative of relatively low within-sample variation. However, in

583    a few of the samples (i.e. 9:00, 21:00) noticeable variation between replicate subsamples was

584    evident. These findings highlight the heterogeneity in marine environments and reinforce the

585    value of replicate subsampling in natural systems.

586    Supplemental Figure 2. Temperature and Salinity plot, with overlaying *in situ* NAC11-7 *ftsZ*

587    gene copies. *ftsZ* copies are proportional to the area of the circles.  The area of the circle

588    representing the 3:00 PM sample corresponds to $1.0 \times 10^4$ gene copies per ml.

589    Supplemental Figure 3. A. Alignment of NAC11-7 FtsZ sequences. B alignment of NAC11-7
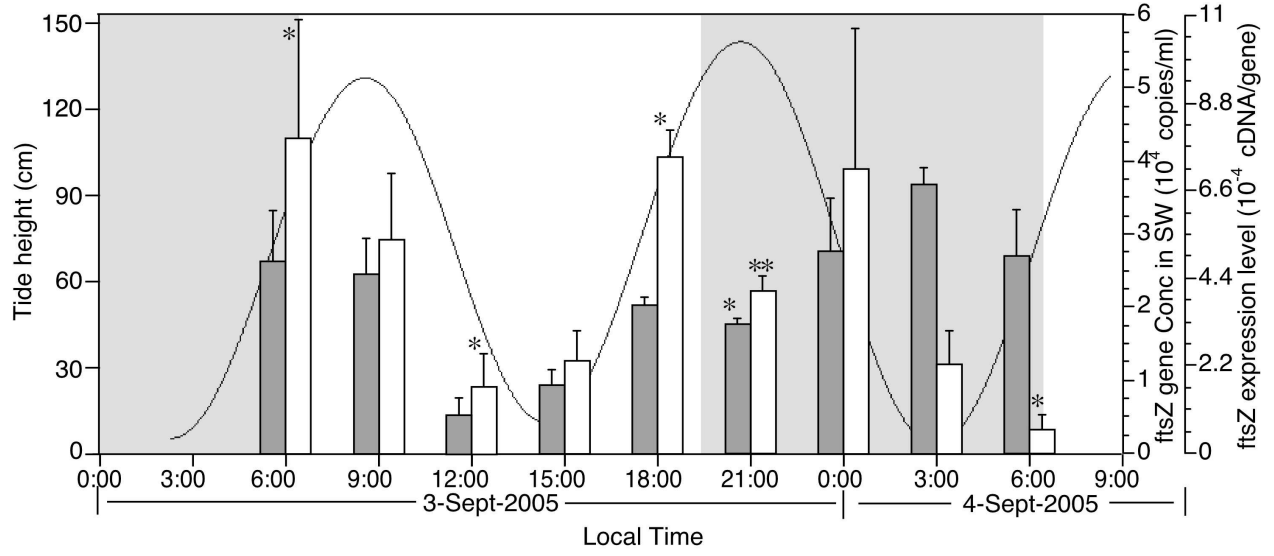
590    *ftsZ* sequences
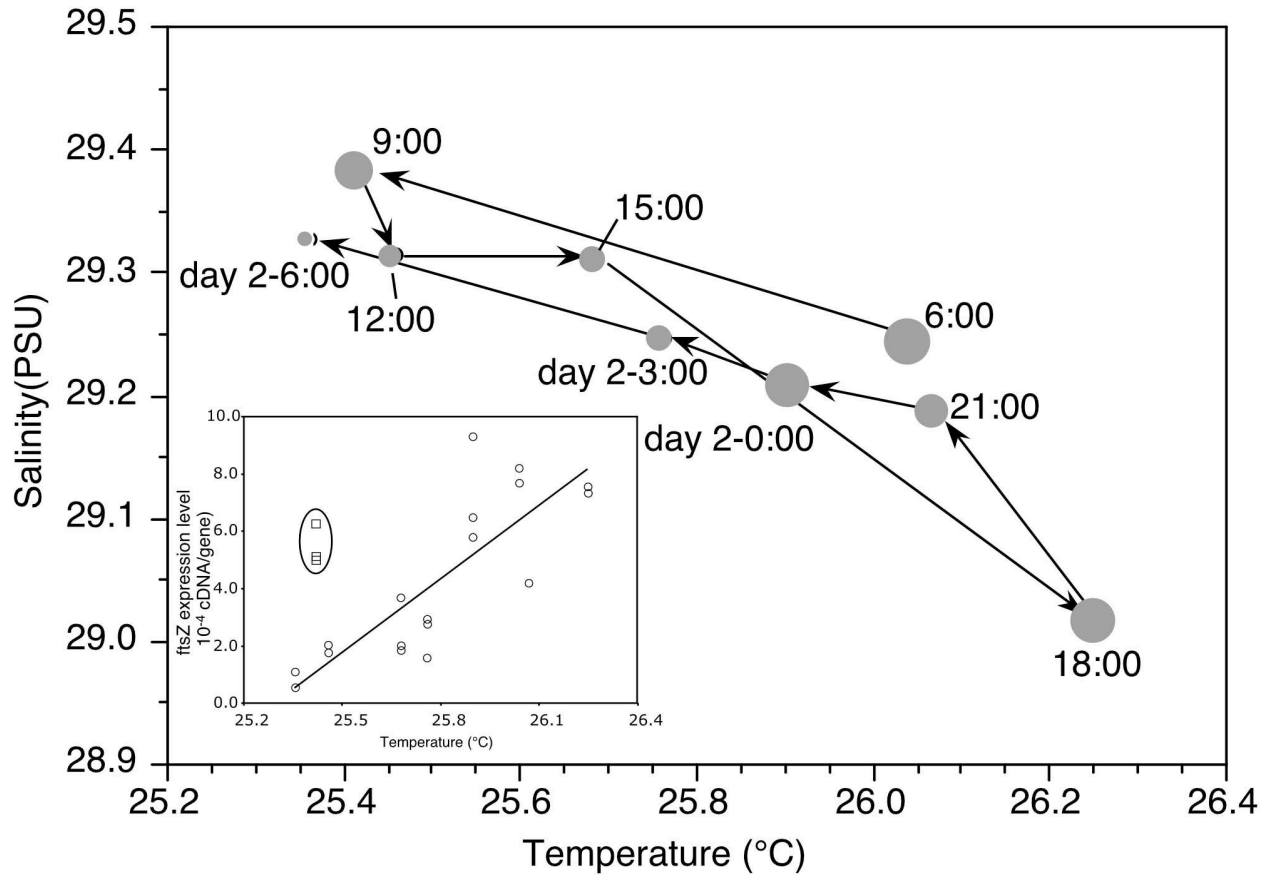
591
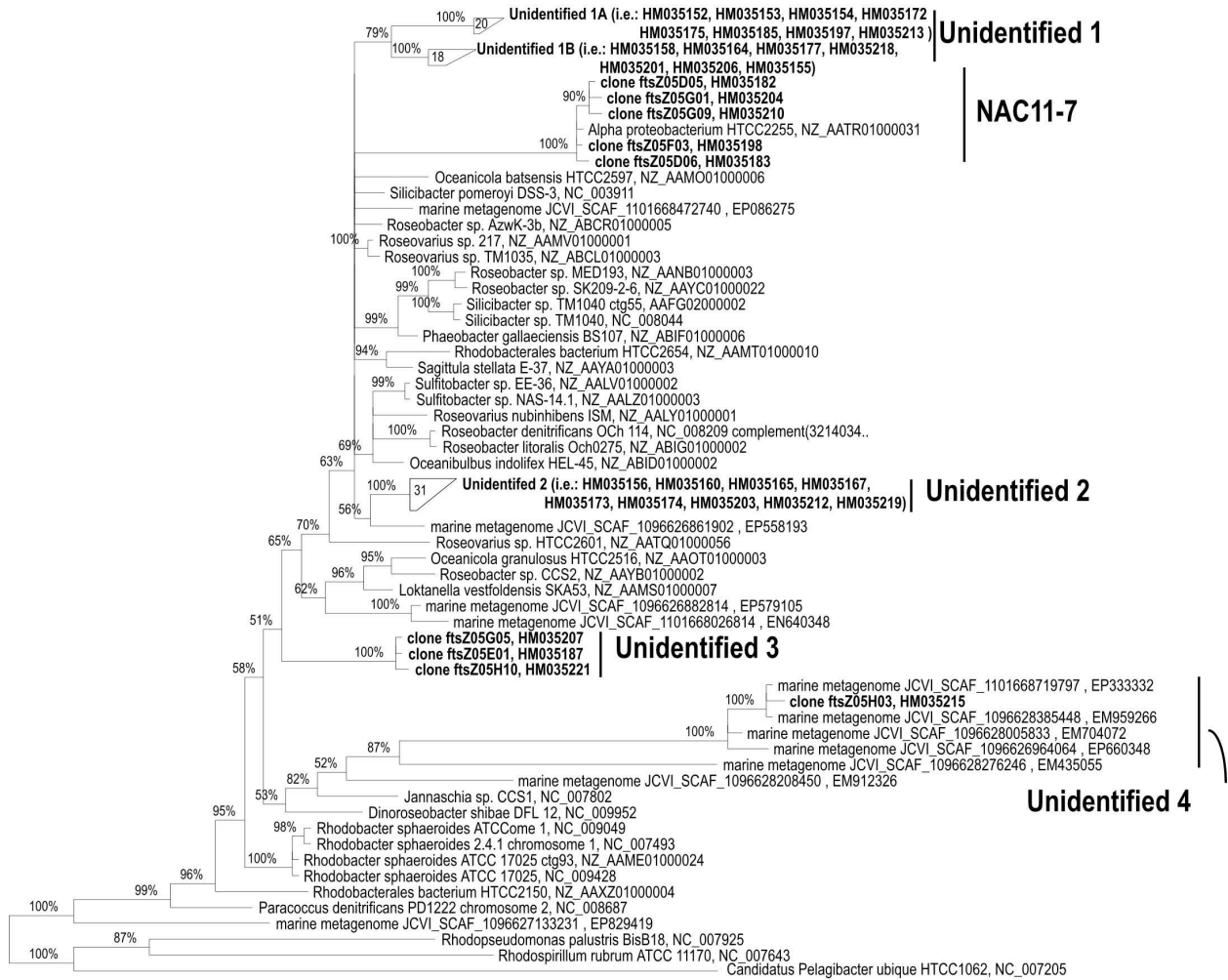
**Figure 1.**

0.10

593    **Figure 2.**



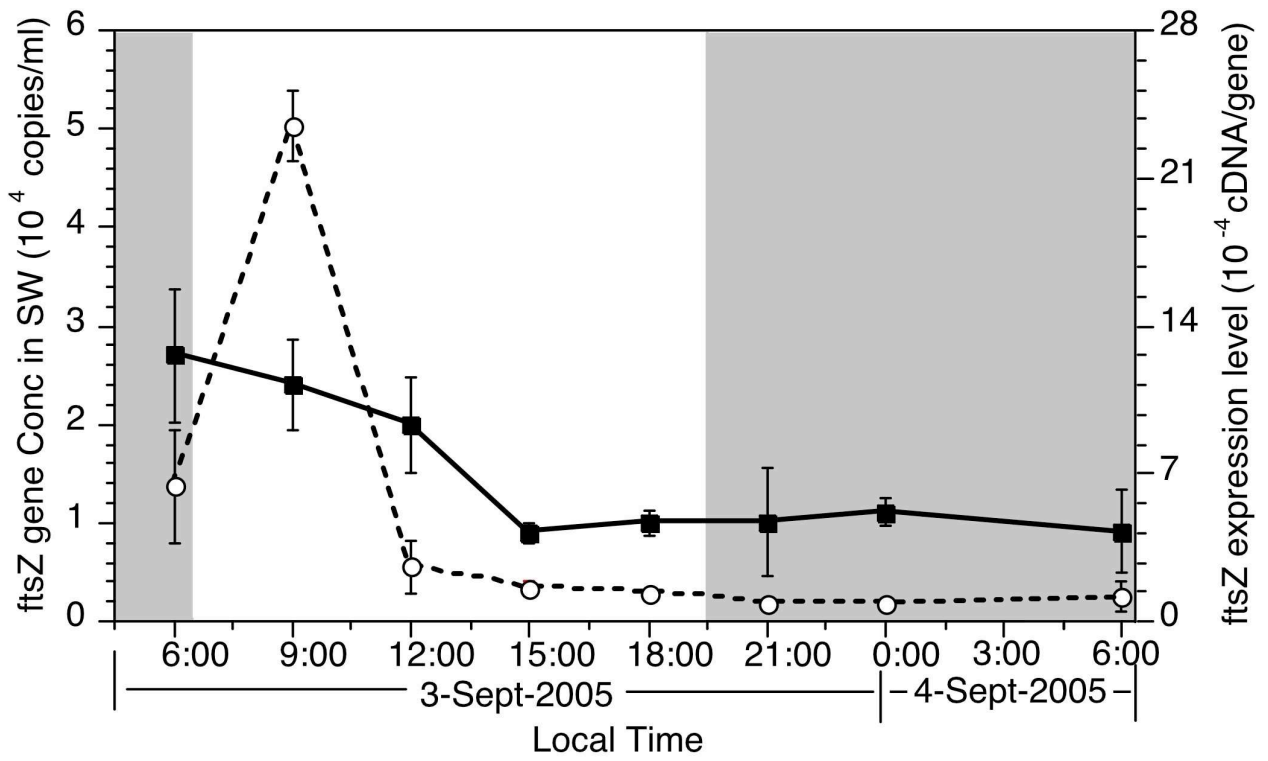594

595

595    **Figure 3.**
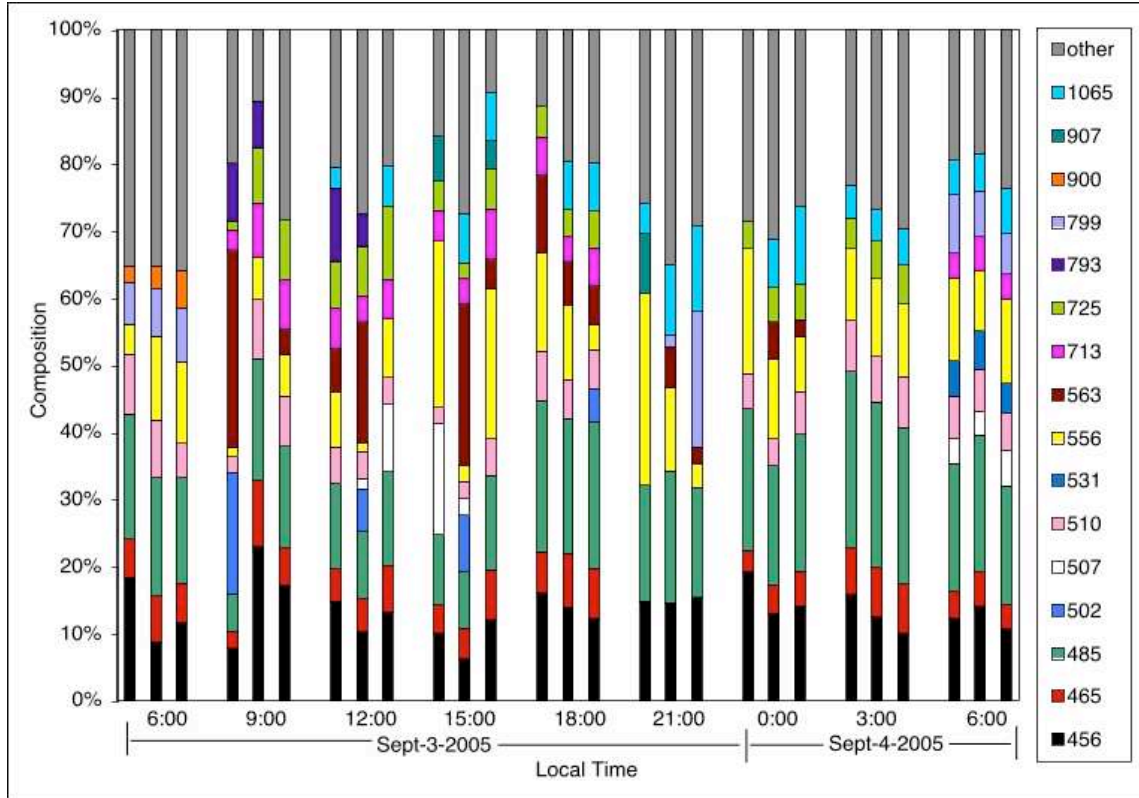


596

597

597    **Figure 4.**
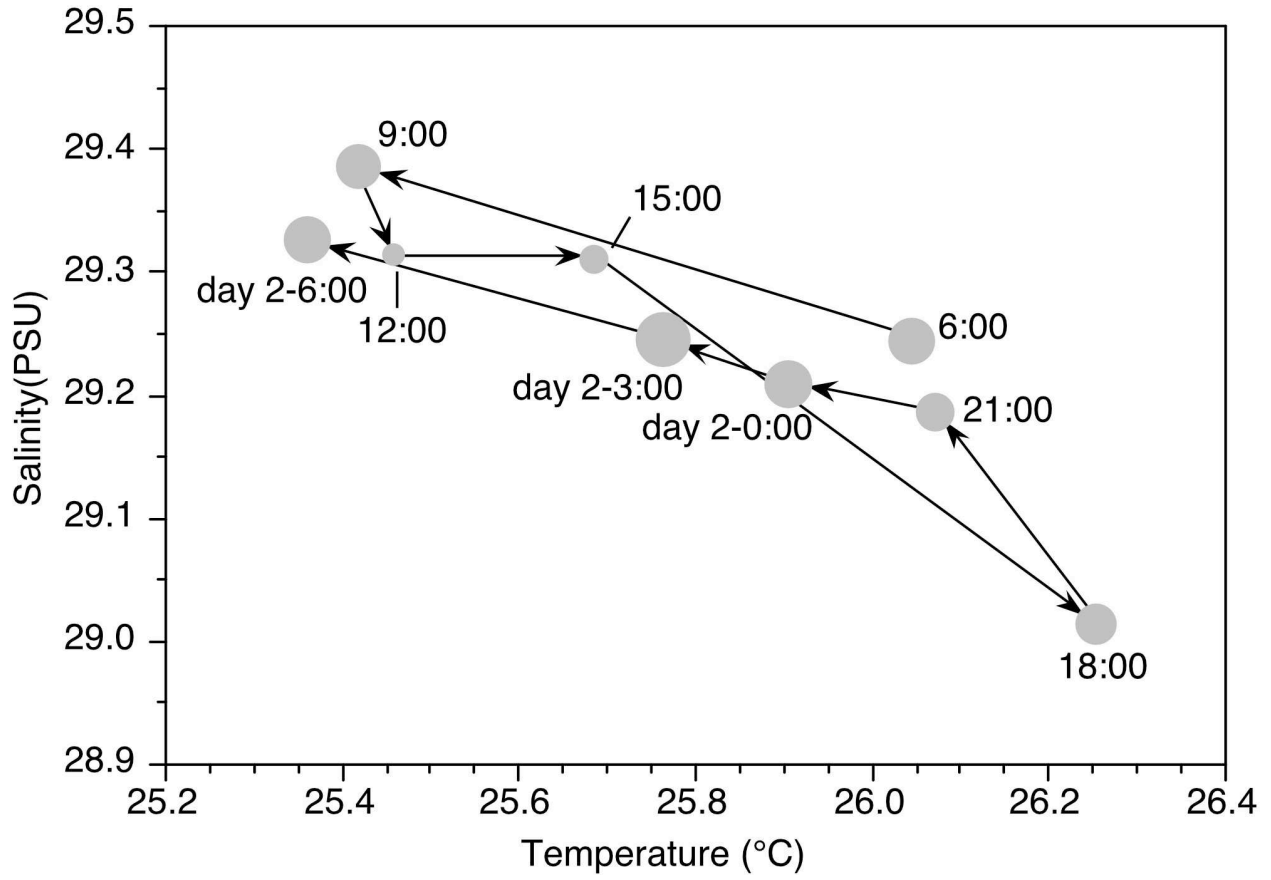
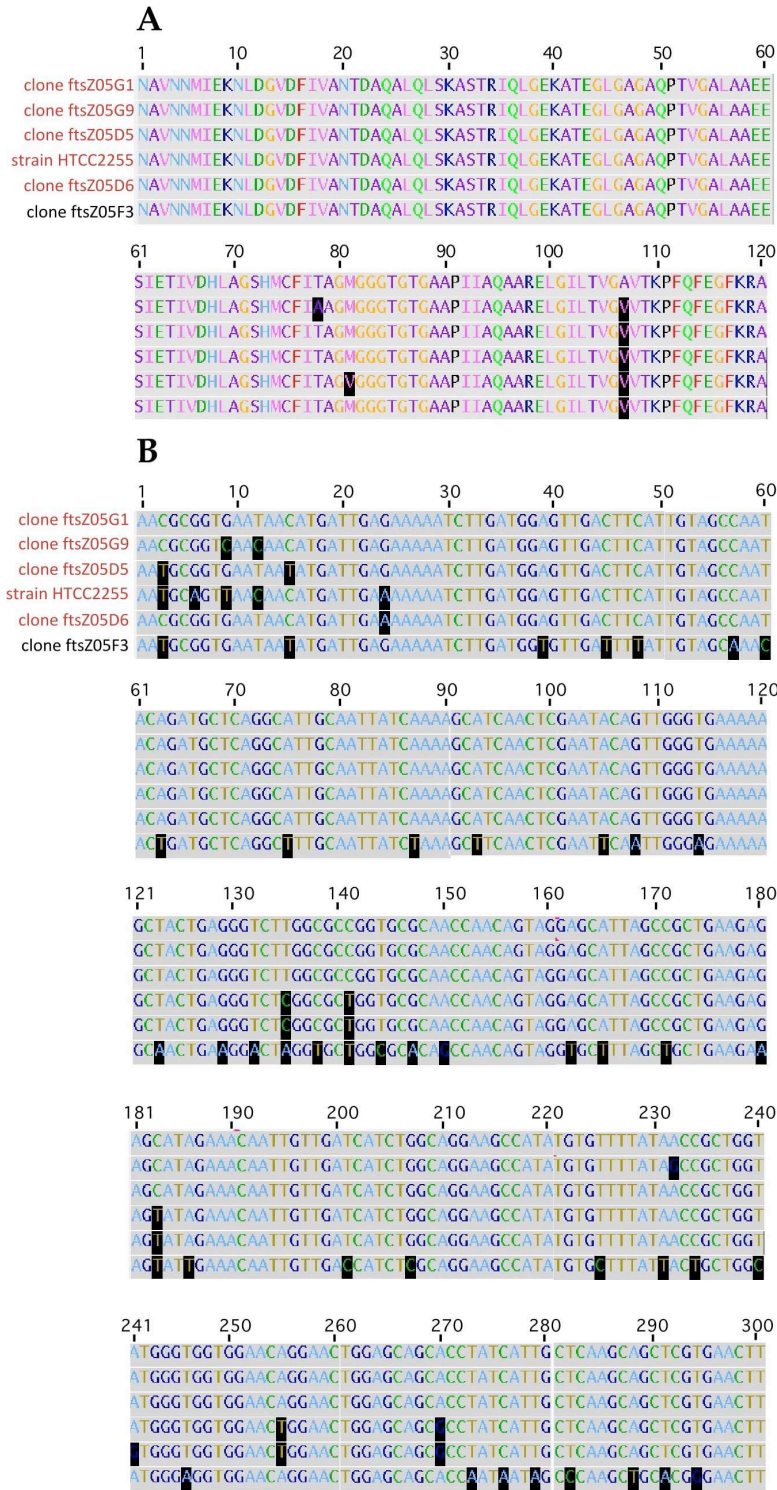

598

599    **Supplemental Figure 1**



600

601

601 **Supplemental Figure 2**



602

603

603     **Supplemental Figure 3**



604