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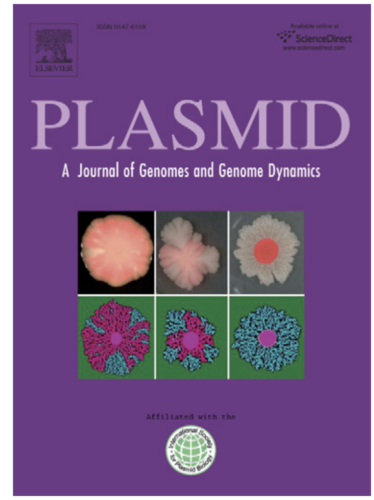

Suite of novel vectors for ectopic insertion of GFP, CFP and IYFP transcriptional fusions in single copy at the *amyE* and *bglS* loci in *Bacillus subtilis*

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Suite of novel vectors for ectopic insertion of GFP, CFP and IYFP transcriptional fusions in single copy at the *amyE* and*bglS*loci in *Bacillus subtilis*

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Abstract

We report the development of a suite of sixintegrative vectors for construction of single copy transcriptional fusions with the *gfpmut3, cfp* and *iyfp* reporter genes in *B. subtilis.* The promoter fusions are constructed using the highly efficient ligation-independent cloning (LIC) technique making them suitable for high-throughput applications. The plasmids insert into the chromosomeby a double cross-over event at the *amyE*or*bglS* loci and integration at each site can be verified by a plate-based screening assay. The vectors allow expression of two different promoters to be determined in the same strain using the *cfp* and *iyfp* reporter genes since CFP and iYFP are spectrally distinct and have comparable half-lives of approximately 2 hours in exponentially growing *B. subtilis* cells. We demonstrate the versatility of these vectors by measuring expression of the *tuaA*and *phoA* operons singularly and in combination, during growth in phosphate limiting conditions.

Keywords: Transcriptional fusions;Fluorescent proteins; Dual labelling;*Bacillus subtilis*.

1. Introduction

The use of transcriptional fusions between promoters and reporter genes is a powerful and proven tool in prokaryotic gene expression studies. While a variety of reporter genes have been utilized, the gene encoding the green fluorescent protein (GFP) from *Aequorea victoria* [1]has emerged as the reporter of choice for many applications. Its widespread usage stems from the fact that GFP detection only requires irradiation with blue light and development of fast-folding GFP variants allow detection within minutes of expression [2, 3]. These featuresallow continuous real time monitoring of transcriptional activity in living bacterial cells with a temporal resolution of minutes [4, 5, 6]. A number of GFP variants engineered for improved brightness and shifted excitation and emission spectrahave been developed [3, 7, 8]: among those are the distinguishable cyan (CFP) and yellow (YFP) fluorescent proteins, which have been employed to visualize differential expression of two genes or localization of two proteins in the same cell [7, 8, 9, 10, 11, 12, 13]. Conveniently, CFP and YFP variants optimized for use in the Gram-positive bacterium *Bacillus subtilis* have been obtained [10, 13, 14, 15].

In this work, we describe a suite of novel vectors designed to generatetranscriptional fusionsbetween *B. subtilis* promoters and the *gfpmut3, cfp* and *iyfp*reporter genes, using the highly efficient ligation-independent cloning (LIC) technique [16, 17, 18]. Each fusion can be integrated into the *B. subtilis* chromosome in single copy at either the *amyE* or the *bglS* locus and correct insertion can be verified using a plate assay. We demonstrate the use and versatility of these vectors by determining theexpression profile of the *tuaA*and *phoA* promoters singularly and in combination in cells grown under phosphate limiting conditions.

2. Materials and methods

2.1. Bacterial strains, plasmidsand growth conditions

The bacterial strains and plasmids used in this study are listed in Table 1. Strain TG1 was used for routine cloning in *Escherichia coli*[19]. Bacterial transformation was carried out by standard procedures: *E. coli* TG1 was transformed by the calcium chloride method [20] and*B. subtilis* strains were transformed as described by Leskela *et al.,* [21]. *E. coli* strains were grown in Luria-Bertani (LB) medium [22], and*B. subtilis* strains were grown in low phosphate defined medium (LPDM) and high phosphate defined medium(HPDM)[23]. Antibiotics were added to cultures when needed at the following concentrations per ml: ampicillin 100 μg; kanamycin 10 μg; chloramphenicol 3μg.

2.2. DNA manipulation and oligonucleotides

DNA manipulations were carried out according to standard procedures as described by Sambrook *et al.* [20]. The oligonucleotides used in this study are listed in Table 2.

2.3. Plasmid construction

Plasmid pBPbglS was constructed by inserting a fragment upstream of*bglS* (amplified with primers oBP231 and oBP232) and afragmentoverlapping the 3'-end of *bglS*(amplified with primers oBP233 and oBP234) either side of the *kan*^{r} cassette in pDG780 [24]. Plasmids pGFPbglS, pCFPbglS and pYFPbglSare derived from pBPbglS by inserting the *gfp*, *cfp* and *iyfp* genes into the unique *Eco*RI site. Specifically, plasmid pGFPbglS contains the LIC-*gfpmut3* cassette that was amplified from plasmid pBaSysBioII [25] with primers oBP237 and oBP238. Plasmid pCFPbglS contains a LIC-*cfp* cassettethat was amplifiedfrom plasmid pDR200[15] using primersoBP354 (containsthe LIC sequence) and oBP355. Plasmid pYFPbglS contains a LIC-*iyfp* cassette amplified from plasmid pIYFP[13] using primersoBP262(contains the LIC sequence) and oBP279.

Plasmid pBPamy is derived from pAC5, generated by amplifying the plasmid backbone using primers oBP275 and oBP276, which excludes the *lac*Zgene reporter[26].Plasmids pGFPamy, pCFPamy and pYFPamyare derived from plasmid pBPamy, constructed by inserting the LIC-*gfp*, LIC-*cfp* and LIC-*iyfp* cassettes (detailed above) into the unique *Eco*RI site.

These plasmids allow ectopic integration of promoter fusionseither at the *bglS* locus, through double homologous recombination at the *bglS* front (427 bp) and *bglS* back (398 bp) sites, or at the *amyE*locus, through recombination at the *amyE* front (537 bp) and *amyE* back sites (1037 bp). A schematic diagram of these plasmids is presented in Figure 1, and the complete sequences are available in the GenBank database, under the following accession numbers:HM204934, pGFPbglS; HM204935, pGFPamy; HM204936, pYFPbglS; HM204937, pYFPamy; HM204938, pCFPbglS; HM204939, pCFPamy.

2.4. Cloning of promoter-containing fragments using the LIC site

To construct plasmids with promoter fusions to the*gfpmut3, cfp* or *iyfp* reporter genes, DNA fragments carrying the promoter regions of the genes of interest, flanked by LIC sequences allowing ligation-independent cloning, were generated by PCR generated and cloned into one of the six newly generated vectorsas described by Botella *et al*[25]. Briefly, the LIC sequence in each vector is TTTTACCGCGGCTTT**CCC GGG**AAGGAGGAACT. Each plasmid is linearized with *Sma*I (sequence in bold above) and treated with T4 polymerase in the presence of dATP for 20 minutes at 22°C followed by 30 minutes at 75°C to inactivate the enzyme - specifically 4 picomoles of vector were treated with 20 units of T4 DNA polymerase in the presence of 1X T4 DNA polymerase buffer and 2.5mM dATP.This generates single-stranded overhangs on either side of the restriction site extending to the underlined A bases. Promoter containing fragments were amplified using oligonucleotideswith a 5' CCGCGGGCTTTCCCAGC 3' tail sequence added to the forward primer and a 5' GTTCCTCCTTCCCACC 3' tail sequence added to the reverse primer. Fragments were then treated with T4 polymerase in the presence of dTTP for 20 minutes at 22°C followed by 30 minutes at 75°C to inactivate the enzyme – specifically 0.2 picomoles of insert were treated with 0.4 units of T4 DNA polymerase in the presence of 1X T4 DNA polymerase buffer and 2.5mM dTTP. This generates inserts with single-stranded ends that are complementary to those of the treated vectors. Treatedplasmids(5 ng)and inserts (15 ng)were mixed with and annealed at room temperature for 10 minutes and transformed into *E. coli*. This procedure results in directional cloning of promoters into the vectors to generate transcriptional fusions.

Promoterregions of *tuaA* and *phoA*(400bp and 514 bp respectively) were amplified using primers pairstuaAF - tuaAR and phoAF - phoAR respectively. Plasmids pBP128 and pBP231 were generated by inserting the *tuaA*promoter region into plasmids pGFPbglS and pCFPbglS respectively. Plasmids pBP186 and pBP192 were generated by inserting the *phoA*promoter region into plasmids pGFPamy and pYFPamyrespectively. All constructs were confirmed by DNA sequencing.

2.5. Construction of B. subtilis strains

B. subtilis strains, listed in Table 1, were obtained by transforming linearized plasmids into recipient strains, giving ectopic insertion at the *amyE* locus or at the *bglS* locus by a double cross-over recombination event. Recombinant plasmids derived from pGFPbglS, pCFGbglS or pYFPbglS were linearized by digesting with *Bam*HI; derivatives of plasmids pGFPamy and pCFPamy were linearized with *Pst*I, while vectors derived from pYFPamy were digested with *Sca*I.

2.6. Verification of integration position

Correct integration at the *amyE* site was tested by patching transformants onto LB agar plates containing 1% (w/v) starch. Strains were grown overnight at 37ºC and stained with a solution containing 0.1% (w/v) potassium iodide and 0.1% (w/v) iodine dissolved in 1N HCl. Lack of amylase activity, indicating correct insertion at the *amyE* site, was visualized as a lack of halo surrounding thecolony (wild type strain 168 was used as a positive control for amylase activity). A similar method was used to testcorrect insertion at the newly developed *bglS* integration site: lack of β-glucanase activity was visualized as a lack of halo surrounding colonies grown on LB agar plates containing 0.4 % (W/V) lichenan (Sigma) and stained with a 0.1% (W/V)Congo redsolution[27].

2.7. Measurement of growth rate and gene expression

*B. subtilis*strainswere grown overnight in HPDM medium at 37ºC and 220 rpm; these cultures were used to inoculate LPDM cultures at a starting OD_{600} of 0.02 in a 96-well plate with optical bottom (Nunc), at a final volume of 100μl per well. Plates were covered with lids to prevent evaporation and incubated in a Synergy™ 2 mutimode microplate reader (Biotek) at 37ºC, set with constant slow shaking for7hours. Growth was monitored turbidimetrically by measuring absorbance at 600 nm. Fluorescence readings were taken by usingthe following filters: excitation 485/20 nm, emission 528/20for GFP; excitation 500/27nm, emission 540/25for IYFP; excitation 420/50 nm, emission 485/20for CFP. Readings were taken from the bottom of the plate, with the exception of CFP readings, which were taken from the top using a 455 nm dichroic mirror.Measurements were taken at 15 minutes intervals. To calculate expression levels, the natural fluorescence of three cultures of wild type *B. Subtilis* strain 168 (containing no reporter gene) were averaged and subtracted from the raw fluorescence value of each reported strain at the same OD_{600} value. Gene expression was then calculated as fluorescence value divided by the OD_{600}at the same time point.

2.8. Measurement of CFP and IYFP half-life

The half-lives of CFP and IYFP were calculated as described by Botella *et al* [25].

3. Results and discussion

3.1 Characteristics of the new vectors

We have constructed a suite of six vectors for generating transcriptional fusions in *B. subtilis*. A schematic diagram of these plasmids is shown in Figure 1. The plasmids combine several features that make them especially useful for high-throughput determination of real-time expression profiles in *B. subtilis*.(1) DNA fragments are cloned into all six plasmids by the same LIC mechanism allowing a choice of both reporter gene (*gfp*, *cfp* and *yfp*) and integration site (*amyE*, *bglS*) for each promoter fusion.The LIC cloning system is highly efficient and amenable to automated cloning technology. (2) Integration occurs by a double cross-over event, ensuring that only one copy of the promoter fusion is present on the chromosome (a promoter fusion integrated by a Campbell-type event can be sometimes present in multiple copies on the chromosome due to amplification or to integration of a dimeric plasmid). Promoter fusions are also stably integrated into the *amyE* and *bglS* chromosomal sites since repeated sequences are not generated during the integration event. (3) These plasmids allow *B. subtilis* to be used as a model system to study heterologous promoters since the required sequences for homologous integration are plasmid encoded. (4) Three fluorescent proteins, GFP, CFP and IYFP are used as reporters of gene expression. A particular advantage is that CFP and IYFP are spectrally distinct and can be used to monitor expression of two different promoters in the same strain. (5) Integration of each reporter fusioncan be made at both the *amyE* and *bglS* loci, from which several advantages accrue: (a) correct integration can be verified by an easy plate screening assay; (b) our development of the *bglS* locus as a site of integration allows these plasmids to be used in conjunction with the multitude of existing plasmids that integrate at the widely used *amyE* locus and (c) expression of two different promoters can be determined in the same strain using the spectrally distinct CFP and IYFP reporters, integrating one fusion at the *amyE* locus and the second at the *bglS* locus. These characteristics confer considerable versatility on the usage of this plasmid suite in expression studies, especially to monitor promoter activity of genes in growing cultures in a high-throughput and automated way with a temporal resolution of minutes.

3.2 Measurement of CFP and IYFP protein half-life in B. subtilis.

Knowledge of the half-life of each reporter protein is required when different reporters are used to comparethe expression profiles of individual promoters. This is especially important when expression of two different promoters is being determined in the same cell, as is possible using the*cfp*- and *iyfp*-containing plasmids reported here. We have previously reported the half-life of GFPmut3 in exponentially growing *B. subtilis* to beapproximately 9 hours [25].Using a similar approach we have determined the half-lives of CFP and IYFP to be approximately 2 hours (two hours and 30 minutes for CFP and two hours and 15 minutes for IYFP,) in exponentially growing *B. subtilis* cells. This makes GFP the reporter of choice in determining promoter activity as described in Botella *et al* [25] since its decay is negligible during short time intervals. However, here we show that CFP and IYFP can both be used to accurately determine the expression profile of different promoters in the same strain since they have comparable half-lives.

3.3Measurement of the expression profiles of the P_{tuaA} and P_{phoA} promoters usingthe pGFPbgls and pGFPamy vectors

The expression profile of two *B. subtilis* promoters was established during growth in phosphate limiting conditions. The *phoA*gene encodes an alkaline phosphatase while the *tuaA* operon encodes teichuronic acid biosynthetic genes and both operons are induced upon phosphate limitation. Plasmids pGFPbgls and pGFPamy were utilized to construct strains BP349 and BP158, which carrythe P_{phoA}*gfpmut3*andP_{tuaA}*gfpmut3* transcriptional fusions integrated at the *amyE*and *bglS*locus respectively. These strains were grown in low phosphate defined medium (LPDM), and expression profiles were determined as outlined in Materials and Methods. Results are shown in Figure 2. Neither fusion is expressed during exponential growth while both are induced at the onset of phosphate limitation, activated by the PhoPR two-component system[28, 29, 30]. Importantly, control strains BP370 and BP381 that havepGFPbglS and pGFPamy without promoters inserted into the chromosome had only background fluorescence levels throughout the growth curve (data not shown). We compared expression of the P_{phoA}*gfpmut3* andP_{tuaA}*gfpmut3* transcriptional fusions at their homologous chromosomal loci with those inserted into the *amyE* (P_{phoA}*gfpmut3*) and *bglS* (P_{tuaA}*gfpmut3*) loci by a double crossover event. Results showed that the profile and level of P_{tuaA}*gfpmut3* expression was almost identical at both chromosomal sites (data not shown). However while the profile of P_{phoA}*gfpmut3* expression was similar at both sites, expression at the homologous site was approximately 3-fold higher that that at the *amyE* site. Differential expression of a particular fusion located at separate chromosomal locations has been previously observed in several studies [31 and references therein]. Thus chromosomal context is important for expression of some transcriptional fusions and must be considered whencomparisons of expression profiles are being made.

3.4Measurement of the expression profile of two different B. subtilis *promoters in the same strain using thepCFPamy and pYFPbgls vectors*

The use of two different integration sites and the spectrally distinguishable cyan (CFP) and yellow (IYFP) fluorescent reporter proteins in this vector suite allowsexpression of two different promotersto be monitored in the same strain. PlasmidpYFPamywas used to construct strain BP355 that has P_{phoA}*iyfp* integrated at the *amyE* locusand plasmid pCFPbglS was used to construct strain BP477 that has P_{tuaA}*cfp* inserted at the *bglS*locus. In addition strain BP543 was generated that has both transcriptional fusions, P_{phoA}*iyfp* integrated at the *amyE* locus and P_{tuaA}*cfp* inserted at the *bglS* locus. Expression of these fusions was measured in the three strains grown in LPDM medium. Results are shown in Figure 3. Only background levels of fluorescence were observed in strains BP371 and BP482 into which the plasmids pCFPbglS and pYFPamy without promoters were inserted (data not shown).The expression profiles of both fusions were similar to that obtained using the GFP reporter protein, although the CFP and IYFP signals are less. This could be due to decreased sensitivity or to the increased turnover of both protein when compared to GFP. Importantlythe expression profiles of both fusionsdetermined in strain BP543 are virtually identical to those obtained in the strainsthat harboured each of the single fusions (Figure 3 A, B). This confirms that the presence of both IYFP and CFP proteins in cells does not interfere with fluorescence measurements of either protein.

Exponentially growing and phosphate limited cells of strains singly carrying the promoter fusions were analyzed by fluorescence microscopy. Only background fluorescence was observed in exponentially growing cells, while a homogeneous population of cells with comparable fluorescence levels was observed in phosphate limited cells (data not shown).

4. Conclusions

We have developeda new suite of vectors for analysis of gene expression using the *gfpmut3, iyfp* and *cfp* reporter genes in *B. subtilis.* Promoter-containing fragments are cloned using the highly efficient LIC system that is amenable to high-throughput automated procedures and the plasmids can integrate at two separate chromosomal loci. We establish that CFP and IYFP proteins have comparable half-lives of approximately 2 hours in exponentially growing cells and demonstrate the versatility of these plasmids by monitoring expression of two different promoters in the same strain.

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Table 1. Bacterial strains and plasmids.

Strain or plasmid	*Genotype*	*Source or reference*
<u>*E. coli* strains</u>		
TG-1	*supE hsd 5 thi (lac-proAB) F'(traD36 proAB lacI^{q}lacZ M15)*	Gibson (1984)
<u>*B. subtilis* strains</u>		
168	*trpC2*	Laboratory stock
BP158	*trpC2 bglS::tuaA-gfpmut3 Kan^r*	pBP128→168
BP349	*trpC2 amyE::phoA- gfpmut3Cm^r*	pBP186→168
BP355	*trpC2 amyE::phoA- iyfp Cm^r*	pBP192→168
BP370	*trpC2 bglS::gfpmut3 Kan^r*	pGFPbglS→168
BP371	*trpC2 bglS::iyfpKan^r*	pYFPbglS→168
BP372	*trpC2 amyE::iyfp Cm^r*	pYFPamy→168

BP381	*trpC2 amyE::gfpmut3 Cm*^{r}	pGFPamy→168
BP477	*trpC2 bglS::tuaA-cfp Kan*^{r}	pBP231→168
BP482	*trpC2 amyE::cfp Cm*^{r}	pCFPamy→168
BP483	*trpC2 bglS::cfpKan*^{r}	pCFPbglS→168
BP543	*trpC2 amyE::phoA-iyfp Cm*^{r}, *bglS::tuaA-cfpKan*^{r}	pBP192→BP477
pBPbglS	Integrative vector for ectopic integrationof DNA contructs by double crossover at the *bglS* locus (Ap^r Kan^r).	This work
pGFPbglS	Integrative vector for the introduction of single copy transcriptional fusions to *gfp* by double crossover at the *bglS* locus (Ap^r Kan^r).	This work
pCFPbglS	Integrative vector for the introduction of single copy transcriptional fusions to *cfp* by double crossover at the *bglS* locus (Ap^r Kan^r).	This work
pYFPbglS	Integrative vector for the introduction of single copy transcriptional fusions to *iyfp* by double crossover at the *bglS* locus (Ap^r Kan^r).	This work
pBPamy	Integrative vector for ectopic integrationof DNA contructs by double crossover at the *amyE* locus (Ap^r Cm^r).	This work
pGFPamy	Integrative vector for the introduction of single copy transcriptional fusions to *gfp* by double crossover at the *amyE* locus (Ap^r Cm^r).	This work
pCFPamy	Integrative vector for the introduction of single copy transcriptional fusions to *cfp* by double crossover at the *amyE* locus (Ap^r Cm^r).	This work
pYFPamy	Integrative vector for the introduction of single copy transcriptional fusions to *iyfp* by double crossover at the *amyE* locus (Ap^r Cm^r).	This work
pBP128	pGFPbglS derivative containing the *tuaA* promoter region (Ap^r Kan^r)	This work
pBP186	pGFPamy derivative containing the *phoA* promoter region (Ap^r Cm^r)	This work
pBP192	pYFPamy derivative containing the *phoA* promoter region (Ap^r Cm^r)	This work
pBP231	pCFPbglS derivative containing the *tuaA* promoter region (Ap^r Kan^r)	This work

Table 2. Oligonucleotides used in this study.

Name	*Sequence (5' to 3')*
oBP231	CGCGGATCCGCTTCACTATTATCGGTTCGTCACCC
oBP232	AAAACTGCAGTCCTGTAACTATCATCATCTTCCCTCTG
oBP233	CCGCTCGAGCTTCACTTACACAGGTCCAACAGATGG
oBP234	CCGCTCGAGGCAGCAGGCTCTTTCACATTTGGC
oBP237	CCGGAATTCTTTTACCGCGGGCTTTCC
oBP238	CCGGAATTC GGTTCTGTAGTCGACTTCTCACC
oBP354	CCGGAATTCTTTTACCGCGGGCTTTCCCGGGAAGGAGGAACTATGGTTTCAAAAGGCGAAGAACTG
oBP355	CCGGAATTCTTACTTATAAAGTTCGTCCATGCC
oBP262	CCGGAATTCTTTTACCGCGGGCTTTCCCGGGAAGGAGGAACTACTATGGATTCA ATAGAAAAGGTAAGC
oBP279	CCGGAATTCGGTTTTCCCAGTCACGACGTTGTAA
oBP275	CCGGAATTCGAAAATTGGATAAAGTGGG
oBP276	CCGGAATTCCCATTATGTACTATCGATCAGACC

tuaAF	CCGCGGGCTTTCCCAGCAATCTTGGAACGAGAGACCG
tuaAR	GTTCCTCCTTCCCACCTTTGTTTGGGATGTTATGATG
phoAF	CCGCGGGCTTTCCCAGCATTTTCCTCCCCAAATGTTA
phoAB	GTTCCTCCTTCCCACCCCGTTTATCATGTTAGGGAA

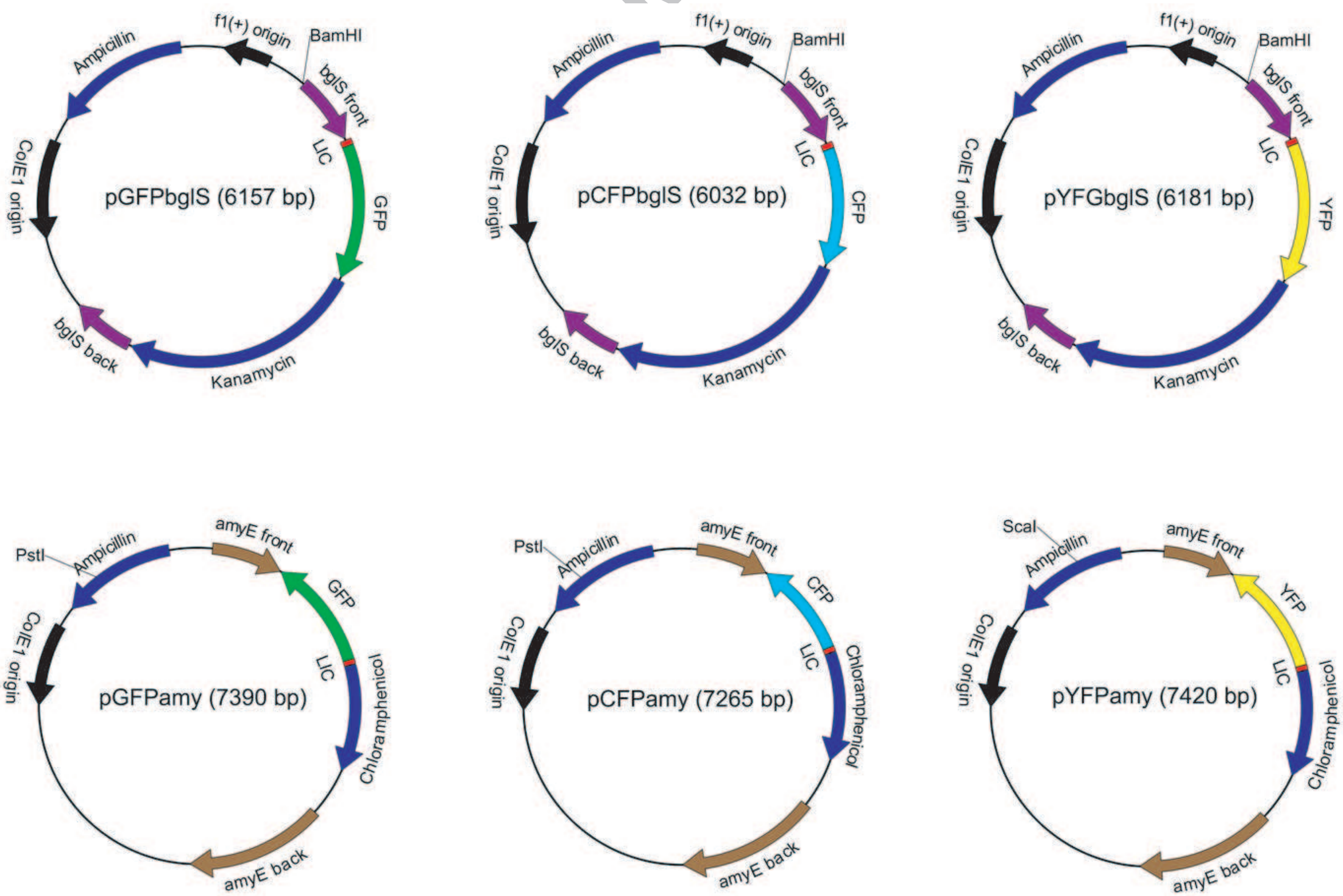
Figure legends

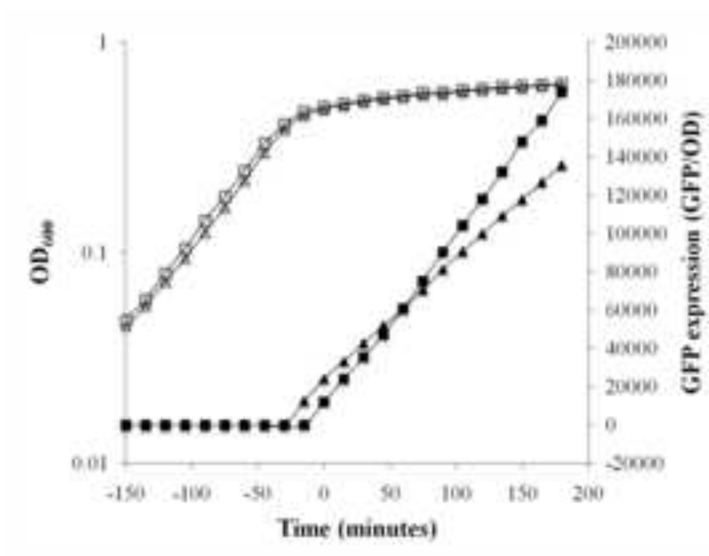
Figure 1. Schematic presentation of plasmids for generating GFP, CFP and IYFP transcriptional fusions at the*amyE* and *bglS* loci using the LIC cloning methodology in *B. subtilis*. Vector names, antibiotic resistance genes and origins of replication are indicated. The reading frames encoding the GFP, CFP and IYFP fluorescent proteins are shown in green, cyan and yellow respectively. The Ligation-Independent-Cloning (LIC) sites for ligation-free cloning of promoters to the fluorescent protein-encoding genes are shown in red. The restriction site used for plasmid linearization is indicated. Integration into the *B. subtilis* chromosome occursvia a double cross-over event at the*amyE* or the *bglS* locus, resulting in selection for chloramphenicol or kanamycin resistance respectively.

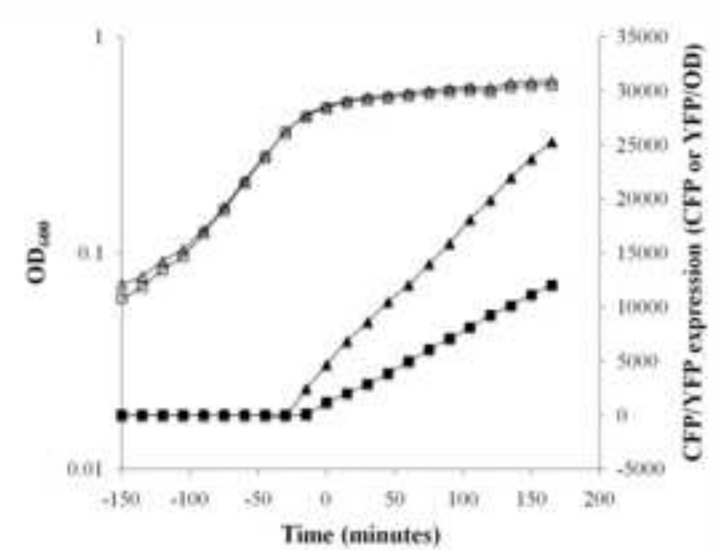
Figure 2. Growth and expressionprofiles of strains carrying transcriptional *gfpmut3* fusions grown in low phosphate defined medium (LPDM). Growth profiles are shown by open symbols and expression by closed symbols: squaresBP349 (*phoA-gfpmut3*); triangles, BP158(*tuaA-gfpmut3*). Time zero indicates the point of transition between the exponential phase and the phosphate-starvation induced stationary phase of growth.

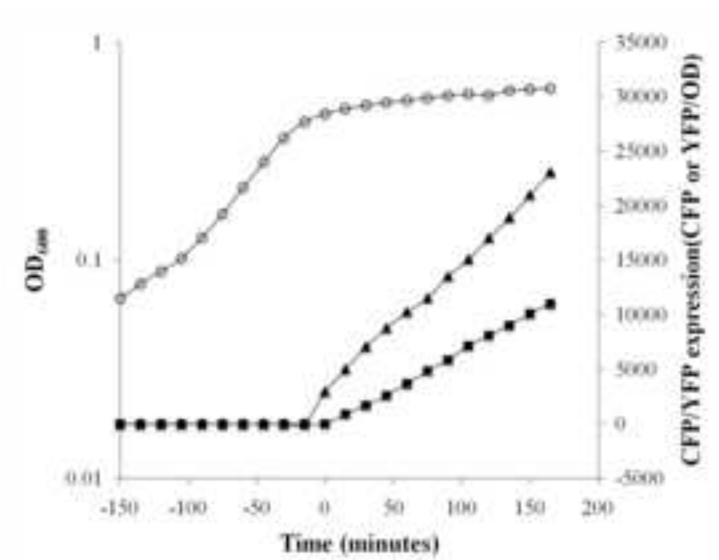
Figure 3. Growth and expression profiles of strains carrying transcriptional fusions to the *iyfp* and *cfp* reporter genes, grown in low phosphate defined medium (LPDM). Growth profiles are shown by open symbols and expression profiles by closed symbols. Time zero indicates the point of transition between the exponential phase and the phosphate-starvation induced stationary phase of growth. A: strain BP355 (*phoA-iyfp*, squares) andstrain BP477(*tuaA-cfp,* triangles). B: strain BP543 (*phoA-iyfp tuaA-cfp*), carrying both

the*phoA-iyfp* (squares) and*tuaA-cfp* (triangles) transcriptional fusions. Open circles indicate growth.


f1(+) origin
BamHI
bglS front
LIC
GFP
Kanamycin
bglS back
ColE1 origin
Ampicillin
pGFPbglS (6157 bp)
f1(+) origin
BamHI
bglS front
LIC
CFP
Kanamycin
bglS back
ColE1 origin
Ampicillin
pCFPbglS (6032 bp)
f1(+) origin
BamHI
bglS front
LIC
YFP
Kanamycin
bglS back
ColE1 origin
Ampicillin
pYFGbglS (6181 bp)
amyE front
GFP
LIC
Chloramphenicol
amyE back
ColE1 origin
PstI
Ampicillin
pGFPamy (7390 bp)
amyE front
CFP
LIC
Chloramphenicol
amyE back
ColE1 origin
PstI
Ampicillin
pCFPamy (7265 bp)
ScaI
Ampicillin
amyE front
YFP
LIC
Chloramphenicol
amyE back
ColE1 origin
pYFPamy (7420 bp)


1
0.1
0.01
OD600
-150
-100
-50
0
50
100
150
200
Time (minutes)
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
-20000
GFP expression (GFP/OD)


1
0.1
0.01
OD600
35000
30000
25000
20000
15000
10000
5000
0
-5000
CFP/YFP expression (CFP or YFP/OD)
-150
-100
-50
0
50
100
150
200
Time (minutes)


1
0.1
0.01
OD600
35000
30000
25000
20000
15000
10000
5000
0
-5000
CFP/YFP expression(CFP or YFP/OD)
-150
-100
-50
0
50
100
150
200
Time (minutes)