Published in final edited form as:

Biotechnol Appl Biochem. 2008 January ; 49(0 1): 35–40. doi:10.1042/BA20070083.

A temperature-sensitive expression system based on the *Geobacillus stearothermophilus* NRS 2004/3a *sgsE* surface-layer gene promoter

Rene Novotny^{*,†}, Harald Berger[†], Thorsten Schinko[†], Paul Messner^{*}, Christina Schäffer^{*}, and Joseph Strauss^{†,1}

^{*}Center for NanoBiotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Gregor-Mendel-Strasse 33, A-1180 Vienna, Austria

[†]Microbial Genomics Unit, Austrian Research Centers and University of Natural Resources and Applied Life Sciences, Vienna, Muthgasse 18, A-1190 Vienna, Austria

Abstract

The *sgsE* gene coding for the S-layer (surface layer) protein in the thermophilic Gram-positive bacterium *Geobacillus stearothermophilus* NRS 2004/3a is strongly induced when the culture is shifted from optimal (55 °C) to maximally tolerable growth temperature (67 °C). Here, we investigated the regulation of the *sgsE* promoter in *G. stearothermophilus* and tested the function of this promoter in *Bacillus subtilis*. We used EGFP (enhanced green fluorescent protein) reporter constructs and found that the *sgsE* promoter has very low basal activity at 28 °C, but is approx. 20-fold induced by elevated growth temperatures (37 and 45 °C). The promoter confers high expression levels, as *EGFP* mRNA levels at 45 °C were approx. 120-fold more abundant than mRNA levels of the *cat* (chloramphenicol resistance) gene, which was transcribed from a constitutive promoter on the same plasmid. In fluorescence-microscopic and Western-blot analysis, the EGFP protein was barely detectable at 28 °C, whereas intermediate and high levels were detected at 37 and 45 °C respectively. The potential to tune expression levels of genes driven by the *sgsE* promoter in *B. subtilis* by simple temperature adjustments presents a considerable potential for its future use as high-yield protein expression system for *B. subtilis*.

Keywords

Bacillus subtilis; enhanced green fluorescent protein (EGFP); expression system; *Geobacillus stearothermophilus*; growth temperature; *sgsE* surface layer (S-layer) gene

Introduction

Two-dimensional crystalline S-layers (surface layers), representing the outermost cell envelope layer of bacteria from all major phylogenetic groups, exist as a regular, closed lattice on the bacterial surface [1,2]. S-layer proteins are among the most abundant cellular

proteins, indicating that these metabolically expensive compounds must indeed play a pivotal role in bacterial life. The biosynthesis of S-layers is a complex process in which the amount of protein synthesis, protein translocation through the cell wall and its incorporation into the existing S-layer lattice have to be co-ordinated with the growth rate of the bacterium and the synthesis of other cell-wall components [3]. Strong expression of S-layer genes generally seems to result from a combination of efficient transcription initiation and usually high mRNA stability [4–7]. All promoter regions described for S-layer genes have so far revealed the conserved hexanucleotide –10 and –35 regions typical of prokaryotic promoters. Studies in various organisms have provided clues to the mechanisms underlying S-layer gene regulation [2]. Frequently, multiple and tandemly arranged promoters are present upstream of S-layer genes [6–9]. For instance, for the cell-wall protein (*cwp*) operon of *Brevibacillus brevis* 47, an organism producing two types of S-layer proteins assembling into two superimposed lattices, three tandemly arranged promoters have been reported. Transcription is initiated growth-phase-specifically from the different promoters; the P2 promoter is used constitutively in *B. brevis* 47 at all stages of growth, whereas the P3 promoter is active only in the exponential phase of growth [8].

We previously cloned the S-layer gene *sgsE* from the thermophilic organism *Geobacillus stearothermophilus* NRS 2004/3a (GenBank® accession no. AF328862) [10] and found that heat stress strongly increases S-layer gene (*sgsE*) steady-state mRNA levels [10]. Under laboratory conditions, the optimal growth temperature of *G. stearothermophilus* NRS 2004/3a is 55 °C [11], whereas the maximum tolerable temperature was shown to be 67 °C.

We have now investigated the promoter of *sgsE* in detail and used the regulatory sequence for gene-expression studies in *Bacillus subtilis* using EGFP (enhanced green fluorescent protein) as a marker protein. Expression levels driven by the *sgsE* promoter are also modulated by the growth temperatures in *B. subtilis*, which shows barely detectable EGFP protein levels at 28 °C, but very strong expression at 45 °C.

Materials and methods

Bacterial strains and growth conditions

G. stearothermophilus NRS 2004/3a was obtained from the N.R. Smith Collection, U.S. Department of Agriculture, Peoria, IL, U.S.A. [11] and was grown on modified S-VIII medium (1% peptone, 0.5% yeast extract, 0.5% meat extract, 0.13% K₂HPO₄, 0.01% MgSO₄ and 0.06% sucrose) to the late-exponential growth phase at 55 or 67 °C. *Escherichia coli* DH5a (Invitrogen) and *B. subtilis* 168 [BGSC (*Bacillus* Genetic Stock Center), Ohio State University, Columbus, OH, U.S.A.; <http://www.bgsc.org/>] were routinely cultivated at 37 °C in LB (Luria–Bertani) medium (Difco). Media were supplemented with chloramphenicol (5 µg/ml), when appropriate. *B. subtilis* 168 cells harbouring plasmid pNW33N-P_{*sgsE*}-EGFP were grown in selective medium (chloramphenicol) overnight and diluted into non-selective medium to an attenuation (*D*) at 600 nm (*D*₆₀₀) of 0.1. After the cultures have reached a *D*₆₀₀ of 0.8, equal amounts of an appropriate dilution were plated on selective and non-selective solid medium. Comparable colony numbers were obtained with and without chloramphenicol for *B. subtilis*.

Standard molecular techniques

Standard molecular techniques followed published procedures [10,12]. Transformation was performed using Subcloning Efficiency DH5 α cells from Invitrogen and *B. subtilis* 168 was transformed by a standard electroporation protocol (Gene-Pulser II apparatus; Bio-Rad).

RNA methods

Total RNA was extracted from *G. stearothermophilus* NRS 2004/3a and from *B. subtilis* 168 by using the RNeasy Mini kit (Qiagen). 5'-RACE (5'-rapid amplification of cDNA ends) from the *sgsE* upstream region of *G. stearothermophilus* NRS 2004/3a RNA was carried out with the second generation 5'-RACE kit (Roche Diagnostics) following the manufacturer's instructions. A 5 μ l portion of the dA-tailed cDNA was used as a template for the first PCR amplification step, using a combination of an oligo dT primer and an *sgsE*-specific primer (SP2). A 1 μ l portion of the resulting sample was used for the second PCR amplification step, using a PCR anchor primer and a nested, specific primer (SP3). PCR mixtures, after reaction, were separated and subcloned into the pGEM-T Easy cloning vector (Promega). *In situ* PCR with a vector-specific primer (M13-47) and an *sgsE*-specific primer (SP4) was used for screening of positive clones, which were subsequently sequenced.

For gene expression analysis, semi-qRT-PCR (quantitative reverse transcriptase PCR) was performed using Platinum SYBR Green QPCR (quantitative PCR) SuperMix-UDG (Invitrogen) and SuperScript III Platinum Two-step qRT-PCR kit with SYBR Green (Invitrogen) and carried out on an iCycler thermal cycler with a MyiQ single-colour real-time PCR instrument as described previously [13]. Results were calculated as the relative amount of product adjusted for the level of internal control amplification (16 S rRNA) obtained for the sample. Primers used for quantification of *sgsE* expression in *G. stearothermophilus* NRS 2004/3a (QA1 to QA6) and for *eGFP* and *cat* transcription (QA7 to QA18) are listed in Table 1.

For the quantification of the plasmid copy number, samples of strains from the late-exponential phase (D_{600} 0.8) were used for plasmid DNA preparation using the Qiagen Plasmid Miniprep kit (DNA recovery up to 99.9%). The concentration of DNA was estimated by using the Quant-iT™ DNA assay kit, Sensitive Range (Invitrogen). The sample total cell numbers were calculated from the amount of genomic DNA determined by using the ChargeSwitch genomic DNA Mini Bacteria kit (Invitrogen) and the calculations are based on a genome size of 4.1×10^6 bp for *B. subtilis*.

In vivo DMS (dimethyl sulfate) footprinting experiments

The procedure essentially followed published protocols [14,15]. Briefly, *G. stearothermophilus* NRS 2004/3a was grown at 55 and 67 °C respectively. After the cells had reached a D_{600} of 0.8, the main culture was separated into 10 ml aliquots, and treated with 1 ml of DMS solution (20 μ l of DMS in 1 ml of 20 mM Mes). The aliquots were further cultured for 10, 20, 40, 60, 90, 120 and 300 s respectively. The methylation reaction was stopped by the addition of 30 ml of TLE-2 [10 mM Tris/HCl, pH 8.0, 1 mM EDTA, 300 mM LiCl and 2% (v/v) 2-mercaptoethanol], followed by DNA extraction, piperidine cleavage and LM-PCR (ligation-mediated PCR). For analysis of the coding strand, approx. 2

μ g of piperidine-cleaved DNA was annealed to the primer FO1 (for all oligonucleotide sequences, see Table 1). Linkers FO2short and FO2long were annealed and ligated to the primer extension products as described in [14]. PCR amplification was carried out with primer FO3 and the long linker primer (FO2long) and for the end-labelling reaction, 10 pmol of [γ - 32 P]ATP-labelled FO4 primer was used. Footprints were analysed by storage phosphor technology by using a Storm 860 PhosphorImager and ImageQuant software (Molecular Dynamics).

Construction of plasmids

A ~0.4 kb fragment corresponding to position -1 to -372 of the *sgsE* upstream region (P_{sgsE} ; GenBank[®] accession no. AF328862) was amplified from *G. stearothermophilus* NRS 2004/3a genomic DNA with primers P1(BamHI) and P2(NcoI). The EGFP fragment was amplified from pEGFPN1 (BD Biosciences Clontech), using primers EGFP(N)_for and EGFP(N)_rev(EcoRI). After digestion with NcoI, P_{sgsE} and the EGFP fragment were ligated. The ligation product served as template for a second PCR amplification reaction using primers P1(BamHI) and EGFP(N)_rev(EcoRI) to give [P_{sgsE} -EGFP]. After digestion with BamHI and EcoRI, the product was ligated into the BamHI/EcoRI-linearized and dephosphorylated plasmid pNW33N (BGSC). The resulting plasmid was named pNW33N- $[P_{sgsE}$ -EGFP] (reporter) and transformed into *B. subtilis* 168. As a negative control, EGFP without P_{sgsE} was cloned into pNW33N using primers EGFP(N)_for(BamHI) and EGFP(N)_rev(EcoRI).

CLSM (confocal laser scanning microscopy)

B. subtilis cells harbouring pNW33N- $[P_{sgsE}$ -EGFP] were grown in LB/chloramphenicol medium at 37 °C to a D_{600} of 0.8, washed with PBS and fixed with 1% agarose in 10 mM phosphate buffer (pH 7.4). The green fluorescence phenotypes of single cells from liquid cultures were detected using a Leica TCS SP2 confocal laser scanning microscope equipped with a detector and a filter set for monitoring GFP. Images were obtained using a confocal-laser-scanning-microscope oil-immersion objective lens (magnification $\times$ 100; numerical aperture 1.40–0.7), and image scanning was carried out with the 488 nm laser line from an Ar/Kr laser and emission was measured between 500 and 555 nm.

SDS/PAGE and Western immunoblotting

For Western blotting, proteins were run on SDS/15%-PAGE gels and transferred on to a Hybond ECL[®] (enhanced chemiluminescence; Amersham Biosciences) nitrocellulose membrane. For the antigen–antibody reaction, membranes were treated with anti-GFP antibodies (Roche Diagnostics) as the primary antibody (dilution 1:5000) and monoclonal goat anti-mouse antibodies conjugated with peroxidase (Jackson ImmunoResearch Laboratories) as the secondary antibody (dilution 1:10000). Blots were exposed on an X-ray film.

Results and discussion

Characterization of the promoter region of the S-layer gene *sgsE*

To determine the transcription start site(s) responsible for *sgsE* gene expression, 5'-RACE was performed using RNA isolated from *G. stearothermophilus* NRS 2004/3a cells at the late-exponential growth phase grown at the optimal growth temperature (55 °C) or at maximum tolerable growth temperature (67 °C). For each growth temperature, two transcription start sites were identified. At the optimal growth temperature, the transcription initiation sites are located 92 bp (P3) and 186 bp (P2) upstream of the translation start site respectively. The promoter motif of P2 at -10 bp upstream of the transcription initiation site shows similarity to the *E. coli* σ^{70} consensus promoter sequence. The switch to 67 °C resulted in the use of alternative transcription initiation sites, which were localized 186 bp (P2) and 221 bp (P1) upstream of the initiating methionine residue. An overview of the 5'-UTR (5'-untranslated region) of *sgsE* and of transcriptional start points is shown in Figure 1.

Nucleotide sequence analysis of the *sgsE* gene upstream region of *G. stearothermophilus* NRS 2004/3a revealed two sets of palindromic sequences (positions 48–67 bp termed IR1 (inverted repeat 1); 122–150 bp termed IR2; positions are relative to the translational initiation site of *sgsE*) potentially capable of forming stem-loop structures (see Figure 1). *In vivo* DMS footprinting analysis (see Supplementary Figure S1 at <http://www.babonline.org/bab/49/bab490035add.htm>) investigating the coding strand for DNA–protein interactions revealed hypersensitive guanine residues at two positions within (position -148) or close to (position -76) the IR sequences. These results suggest the action of a transcription factor at these sequences in response to different growth temperatures. It is well established that hypersensitive purine residues in DMS-treated DNA result from local distortions in the DNA helix, and these secondary-structure changes might have effects on protein–DNA interactions of regulators [16]. Hypersensitive purine residues inside and near inverted repeats of the *sgsE* promoter in fact indicate such structural changes when cultures are shifted from optimal to temperature-stress conditions.

Expression of *eGFP* under the control of the *sgsE* promoter region in *B. subtilis*

G. stearothermophilus NRS 2004/3a is not amenable to genetic transformation. We therefore expressed the EGFP under the control of the *sgsE* 5' regulatory sequence in *B. subtilis*. A fragment comprising 0.4 kb of the promoter region including the *sgsE* initiation codon was cloned in frame with the sequence encoding EGFP into the *E. coli/B. subtilis* shuttle vector pNW33N, which confers chloramphenicol resistance (see the Materials and methods section for details). Constructs omitting the 0.4 kb *sgsE* 5'-UTR were designed as controls. Stability of the expression plasmids over several generations was investigated by comparison of colony numbers obtained from cultures grown under selective or non-selective conditions (for details, see the Materials and methods section). We found that the plasmids were highly stable in *B. subtilis* for at least 50 generations (results not shown).

sgsE promoter activity is modulated by the growth temperature in *B. subtilis*

We examined the expression level of *eGFP* under the control of the *sgsE* promoter in *B. subtilis* and whether this level is influenced by the growth temperature. Overnight cultures

(37 °C) were separated into aliquots and further incubated at standard (37 °C), elevated (45 °C) or decreased (28 °C) growth temperature. Cultures were harvested at D_{600} 0.8, and all subsequent analyses were performed with the same culture material. Aliquots of this material were used to determine *eGFP* mRNA levels and plasmid copy numbers by QPCR, and EGFP protein levels by Western blotting.

sgsE-driven *eGFP* expression was quantified by qRT-PCR using cDNA of 16S rRNA to normalize input. The expression levels of *eGFP* in the different cultures obtained by this method were then compared with the 16S rRNA-normalized expression levels of the *cat* gene, which confers chloramphenicol resistance and is constitutively expressed from the same pNW33N plasmid backbone. Transcript levels were low at 28 °C and continuously increased with raising temperature, resulting in an approx. 20-fold higher transcript level at 45 °C (Figure 2). Expression levels at 45 °C are approx. 120-fold stronger than constitutive *cat* resistance gene mRNA levels. In order to exclude that different transcript levels of *eGFP* originated from different plasmid copy numbers, we correlated relative *eGFP* expression levels with plasmid copy numbers under different growth temperatures. A constant number of approx. 30 copies per cell over the whole range of growth temperatures was observed (results not shown). The presence or absence of [*P_{sgsE}-eGFP*] on the plasmid had negligible influence on plasmid copy number.

We then examined EGFP protein levels at different growth temperatures by fluorescence microscopy and Western-blot analysis. Figure 3 shows the fluorescence-microscopy images. Control cells harbouring promoter-less EGFP plasmids showed some background fluorescence level (Figure 3A) and similar levels were observed at low temperatures (28 °C, Figure 3B). In contrast, markedly enhanced fluorescence of *B. subtilis* cells containing [*P_{sgsE}-eGFP*] was detected at elevated temperatures (37 and 45 °C, Figures 3C and 3D respectively). The differences in protein levels are even more evident in Western-blot analysis (Figure 4). Only trace amounts of EGFP could be detected in cultures grown at 28 °C, which corroborates microscopical observations and confirms tightness of the *sgsE* promoter in *B. subtilis* under these conditions. In contrast, intermediate and high EGFP levels were detected in cultures grown at 37 and 45 °C respectively. Protein-expression data hence are in good agreement with *eGFP* mRNA levels. *B. subtilis* strains containing the *P_{sgsE}-eGFP* expression plasmid showed normal growth kinetics at 28 and 37 °C, and even enhanced cell growth at 45 °C, when compared with pNW33N-containing strains (results not shown).

The *sgsE* promoter-based expression system hence shows remarkable features, making it well suitable for future applications in protein-expression projects: (i) minimal background expression under OFF conditions and highly inducible (~20-fold) under ON conditions; (ii) easy switch between OFF–ON status by simple temperature adjustments, (iii) no chemical inducer/repressor required; and (iv) high expression level under ON conditions (~120-fold higher *eGFP* mRNA levels compared with the chloramphenicol resistance marker *cat* gene). Minimal background expression and strong inducibility by simple temperature shifts combined with high levels of expression presents a considerable potential for the future use of this system in gene expression studies and high-yield protein production in a Gram-positive bacterium.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the BGSC for provision of plasmid pNW33N. Work at the Center for NanoBiotechnology was supported by Austrian Science Fund grants P15612-B10 and P18013-B11 (to P. Messner) and P19047-B12 (to C. Schäffer) and work in the laboratory of J. S. was supported by the Austrian Science Fund grants P17018.

Abbreviations used

BGSC	<i>Bacillus</i> Genetic Stock Center
CLSM	confocal laser scanning microscopy
DMS	dimethyl sulfate
EGFP	enhanced; green fluorescent protein
IR	inverted repeat
LB	Luria–Bertani
qRT-PCR	quantitative reverse transcriptase PCR
RACE	rapid amplification of cDNA ends
S-layer	surface layer
UTR	untranslated region

References

1. Sleytr UB, Messner P. Annu. Rev. Microbiol. 1983; 37:311–339. [PubMed: 6416145]
2. Bahl H, Scholz H, Bayan N, Chami M, Leblon G, Gulik-Krzywicki T, Shechter E, Fouet A, Mesnage S, Tosi-Couture E, et al. FEMS Microbiol. Rev. 1997; 20:47–98. [PubMed: 9276928]
3. Sleytr UB, Beveridge TJ. Trends Microbiol. 1999; 7:253–260. [PubMed: 10366863]
4. Fisher JA, Smit J, Agabian N. J. Bacteriol. 1988; 170:4706–4713. [PubMed: 3049545]
5. Sleytr UB, Bayley H, Sara M, Breitwieser A, Kupcu S, Mader C, Weigert S, Unger FM, Messner P, Jahn-Schmid B, et al. FEMS Microbiol. Rev. 1997; 20:151–175. [PubMed: 9276930]
6. Boot HJ, Kolen CP, Andreadaki FJ, Leer RJ, Pouwels PH. J. Bacteriol. 1996; 178:5388–5394. [PubMed: 8808926]
7. Kahala M, Savijoki K, Palva A. J. Bacteriol. 1997; 179:284–286. [PubMed: 8982011]
8. Adachi T, Yamagata H, Tsukagoshi N, Udaka S. J. Bacteriol. 1989; 171:1010–1016. [PubMed: 2914862]
9. Jarosch M, Egelseer EM, Mattanovich D, Sleytr UB, Sara M. Microbiology. 2000; 146:273–281. [PubMed: 10708365]
10. Novotny R, Schaffer C, Strauss J, Messner P. Microbiology. 2004; 150:953–965. [PubMed: 15073305]
11. Messner P, Hollaus F, Sleytr UB. Int. J. System. Bacteriol. 1984; 34:202–210.
12. Sambrook, J.; Fritsch, EF.; Maniatis, T. Molecular Cloning: A Laboratory Manual. 2nd edn. Spring Harbor Laboratory; Cold Spring Harbor, NY: 1989.
13. Bernreiter A, Ramon A, Fernandez-Martinez J, Berger H, Araujo-Bazan L, Espeso EA, Pachlinger R, Gallmetzer A, Anderl I, Scazzocchio C, Strauss J. Mol. Cell. Biol. 2007; 27:791–802. [PubMed: 17116695]

14. Wolschek MF, Narendja F, Karlseder J, Kubicek C, Scazzocchio C, Strauss J. Nucleic Acids Res. 1998; 26:3862–3864. [PubMed: 9685506]
15. Mueller PR, Wold B. Science. 1989; 246:780–786. [PubMed: 2814500]
16. Saluz HP, Jost JP. Crit. Rev. Eukaryot. Gene Expression. 1993; 3:1–29.

Figure 1. Nucleotide sequence of the *sgsE* 5'-UTR in *G. stearothermophilus* NRS 2004/3a
Promoters P1/P2 activated in cells grown at 67 °C and promoters P2/P3 activated in cells grown at 55 °C with their -10 and -35 recognition sequences (boldface) are indicated. Transcriptional start points (TSP) are indicated by arrows. RBS, ribosomal binding site. Light grey, IRs (inverted repeats); mid grey, -10 and -35 boxes of promoter P1; dark grey, -10 and -35 boxes of promoters P2 and P3.

Figure 2. qRT-PCR analysis of *eGFP* gene expression in *B. subtilis* cells containing a control plasmid lacking the *sgsE* promoter (negative control, neg.) or containing the *sgsE* promoter in front of the *eGFP* coding sequence (P-eGFP)

Cells were pregrown at 37 °C to D_{600} 0.6. Aliquots of the cultures were diluted to D_{600} 0.1 in growth medium and further incubated at 28, 37 or 45 °C until they reached D_{600} 0.8.

Equal amounts of RNA were used for transcriptional analysis (for experimental details and normalization of expression levels to cell density and plasmid copy numbers, see the Materials and methods section). *eGFP* expression levels (expressed as arbitrary numbers on the ordinate axis) were obtained from the normalization procedure.

Figure 3. CLSM images of *B. subtilis* 168 cells grown at different temperatures

Cells either contained a control plasmid lacking the *sgsE* promoter in front of *eGFP* (A) or contained a plasmid expressing *eGFP* under the control of the *sgsE* promoter (B–D). (A, D) Cells grown at 45 °C; (B, C) cells grown at 28 and 37 °C respectively. For each panel, a fluorescent picture (left part) and the corresponding non-fluorescent image (right part) are shown. Scale bar, 5 μ m.

Figure 4. Western-blot analysis of EGFP expression

B. subtilis cells either containing control constructs (lanes 1–3) or *sgsE_p-eGFP* constructs (lanes 4–6) were pregrown at 37 °C to D_{600} 0.6. Aliquots of the cultures were diluted to D_{600} 0.1 in growth medium and further incubated at 28 °C (lanes 1 and 4), 37 °C (lanes 2 and 5) or 45 °C (lanes 3 and 6) until they reached D_{600} 0.8. Equal amounts of protein extracts (20 μ l of extract; 0.35 mg/ml) were loaded on to the gels. One gel was subjected to Western-blot analysis using anti-GFP antibodies (upper panel) and the second gel was Coomassie Brilliant Blue-stained (lower panel). Strong signals in upper panel (lanes 4 and 5) at ~27 kDa represent EGFP. The values on the right are molecular masses in kDa

Table 1

Primers used in the cloning procedure, characterization of the transcription start site (SP), *in vivo* footprinting (FO) and qRT-PCR amplification reactions (QA)

Oligonucleotide name	Nucleotide sequence (5'→3')
P1(BamHI)	AATCAGGATCCTGTTTTTGACAAAATGTTTGCC
P2(NcoI)	AATCACCATGGAGCCTAAAATCCCCCTTCG
EGFP(N)_for	GGTGATGCGTTTTTGGCAGTAC
EGFP(N)_rev(EcoRI)	AATCAGAATTCGGACAAACCACAAGTGAATGCAG
EGFP(N)_for(BamHI)	AATCAGGATCCATGGTGAGCAAGGGCGAGG
SP1	ACGCTTGTTTTGCTTTGTG
SP2	TTTCGCTTGGCTGACGAC
SP3	CTGGCTGTGGCAAGTTTGA
SP4	CCATAAAGCCTAAAATCCCCCTTCG
M13-47	CGCCAGGGTTTTCCAGTCACGAC
FO1	CTGGCTGTGGCAAGTTTGA
FO2short	GAATTCAGATC
FO2long	GCGGTGACCCGGGAGATCTGAATTC
FO3	TTTTGTCCATAAAGCCTAAAATCCCCC
FO4	CCATAAAGCCTAAAATCCCCCTTCG
QA1 (cDNA_sgsE_rev)	CTACATTATTATCGGTAATCGCGCCTG
QA2 (DS/for6)	ACGCACGTCATTACGATCAG
QA3 [sgsE(RNA)_rev]	GGACAGCGTAATCGGTTTAGAC
QA4 (cDNA_16S_rev)	GTCTGGGCCGTGTCTCAGTCCC
QA5 (16S_forward)	AGAGTTTGATCCTGGCTCAG
QA6 (16S_revB)	GTCGCCTTGGTGAGCCG
QA7 (cDNA_cat_rev)	GCCAGTCATTAGGCCTATCTGAC
QA8 (RT-PCR_cat_for)	GGTTCGGGGAAATTGTTTCCC
QA9 (RT-PCR_cat_rev)	CCTGCATGATAACCATCACAAC
QA10 (cDNA_EGFP_rev)	CAGGGCGGACTGGGTGCTCAGG
QA11 (RT-PCR_EGFP_for)	CAGGAGCGCACCATCTTCTTC
QA12 (RT-PCR_EGFP_rev)	GCTGCCGTCCTCGATGTTGTGG
QA16 [cDNA_16S(B. subtilis)_rev]	CGTGGTGTGACGGGCGGTGTGTAC
QA17 [RT-PCR_16S(B. subtilis)_for]	GGTGGTGCATGTTGTCTGTCAGC
QA18 [RT-PCR_16S(B. subtilis)_rev]	GCGATCCGAACTGAGAACAG