Supplemental material

Slide preparation method (in detail)

1. Clean two microscope glass slides (e.g. Knittel Glass, 7.6 x 2.6 cm,) with 70% ethanol and water.
2. Take a gene frame (ABgene; 1.7 x 2.8 cm) and carefully remove one of the plastic foils from the gene frame without causing disassembly of the plastic cover on the other side of the gene frame. Attach the gene frame in the middle of one of the glass slides by first facilitating contact on just one side, followed by guided attachment of the remaining gene frame with a fingernail. Prevent air bubbles while attaching the gene frame to the glass slide.
3. Prepare a similar gene frame glass slide and place this one in a sterile petridish at 30 °C to pre-warm the slide.
4. Supplement time-lapse CDM (20) with maltose sporulation medium (MSM) (8) in a 1:50 dilution.
5. Mix 1.5 ml of this medium with 500 µl 40% polyacrylamide (PAA) solution (Serva, acrylamine/BIS 37.5:1). Add 20 µl 10% ammonium persulphate (APS) and 2 µl TEMED and then immediately pour 500 µl of this liquid within the gene frame on the prepared microscope glass slide. Make sure the whole area, including the borders, is fully covered.
6. Place the second glass slide on the PAA-filled gene frame. Try to avoid air bubbles. Leave the prepared slide at room temperature for 30 minutes to allow for polymerization.
7. After polymerization, remove the upper glass slide carefully and cut the PAA patch in six equal-sized strips using a sterilized scalpel. Carefully place the slices in sterile mQ

water in a petridish and wash the strips for 30 minutes on a shaking table. Repeat this washing step with fresh mQ for another 30 minutes and then replace the mQ water with CDM + 1:50 MSM for the final wash step. Continue the washing of the slices until the cells are ready.

8. Take the pre-warmed glass slide and place up to three strips between the gene frame, making sure there are gaps on either side of the slice. Due to some swelling during the washing, cutting of the strips to fit within the gene frame may be necessary. Remove the second protective plastic layer from the gene frame.
9. Now add 1.5 µl of prepared cells (see section 'culture preparation') onto a PAA strip: Load single cells on the solid medium without touching it with the pipet tip. Always start on top of the PAA patch and allow the liquid to disperse equally on its assigned growth area by turning the slide up and down. The slide is ready, as soon as the edges of the liquid become corrugated and movement of the liquid is no longer visible when turning the slide.
10. Place a clean microscope slide cover slip (24 x 50 mm) on the gene frame from one side to the other (avoid air bubbles). Assure complete attachment by applying pressure on the cover slip along the gene frame with your fingernail. If the cover slip is placed on the cells without allowing them to dry long enough, cells tend to grow on top of each other during the experiment. Also be careful not to wait too long before applying the cover slip, since the PAA will then be too dry.
11. Immediately search for single cells using an inverted microscope with prewarmed climate chamber and time-lapse microscopy software. Make sure to select cells that are relatively close to the periphery of the PAA slice, as oxygen distribution in the middle of the slice is poor and hampers growth and development of the cells and also negatively affects fluorescence.

Table S1 Primers used in this study.

Primer name	Sequence	Properties
Bce14_cD800HindIII-F	CCCAAGCTTGACAAAAATAGACAGATACTCC	HindIII
Bce14_cDpstI-R	GCATTGGTTCTGCAGATGCATAGTTTTTTCTCTCCTC	PstI, includes the first two codons of *cotD*
TIFN73	CGCGGGTACCGATGGGAAATACGGTTTAATCG	KpnI
TIFN74	CGCGTCTAGACTTTTTATTAAAATAAAACTTCCGATTGTTTTTC	XbaI
TIFN75	CGCGGGTACCGATGAATACAGCTGGACAAATG	KpnI
Bce14-upctD-Xba-R	GCTCTAGACTCTCGTTAAAAAAATCTGATTACAC	XbaI
TIFN77	CGCGGGTACCGATTTCACAGTCATACATTTCGCG	KpnI
TIFN78	GCGCTCTAGACGGGAGTTGCTGTACAGTG	Xba

Figure S1

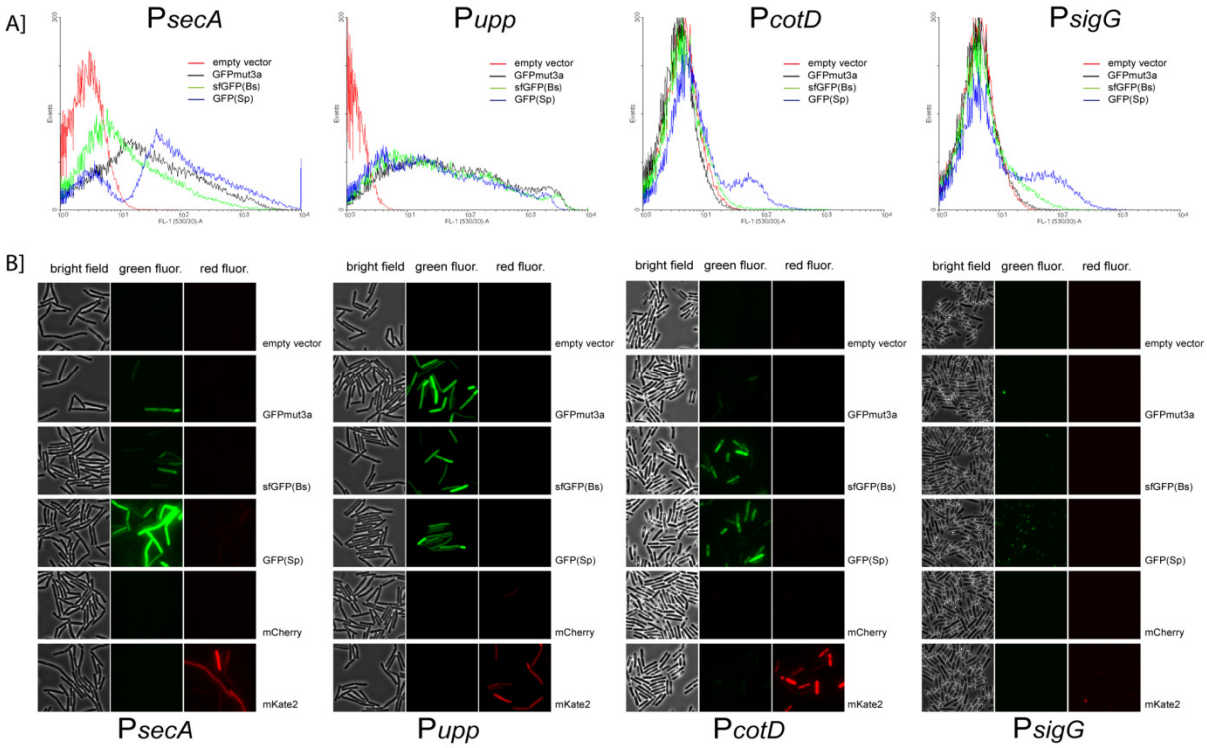

Figure S1 Fluorescence signal of various FP variants in *B. cereus* ATCC14579. Three GFP variants (GFPmut3a, sfGFP(Bs) and GFP(Sp)) and two RFP variants (mCherry and mKate2) were expressed from multicopy pAD123-derived vectors driven by four different promoters

(P_{secA}, P_{upp}, P_{cotD} and P_{sigG}). **A]** The fluorescence intensity and distribution of the various GFPs was monitored using flow cytometry analysis as described in the Materials and Methods section. The fluorescence intensity (in arbitrary units) is shown on the X-axis against the number of events (max 50,000) on the Y-axis. As a negative control, the empty vector pAD123 was used (no fluorescent signal, represented by the red line). GFPmut3a is represented by the black line, sfGFP(Bs) by the green line and GFP(Sp) by the blue line. **B]** The fluorescence intensity and distribution of the various GFPs and RFPs was monitored using fluorescence microscopy analysis, as described in the Materials and Methods section. Arbitrary pictures taken in the bright field channel, the green fluorescence channel (FITC) and the red fluorescence channel (TRITC) are shown for each promoter.