Supporting Information

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SI Materials and Methods

Bacterial Strains, Plasmids, and Growth Conditions. The bacterial strains and plasmids used are listed in Tables S1 and S2. The *Escherichia coli* strains were routinely grown in Luria–Bertani agar (LBA) (EMD Chemicals Inc.) at 37 °C for 48 h and Terrific Broth (TB) (Tecknova) at 37 °C with aeration and vigorous shaking (approximately 300 rpm) for 4–6 h. One Shot® TOP10 *E. coli* strain (Invitrogen), does not require Isopropyl β-D-1-thiogalactopyranoside (IPTG) to induce expression form the LacZ promoter. BL21 Star™ (DE3) pLysS One Shot® *E. coli* strain (Invitrogen) requires IPTG (0.5 mM in broth) to induce optimal expression of recombinant fluorescent proteins (FPs). For genomic DNA extraction, the strains were grown overnight and were then maintained as frozen stocks at −80 °C in TB containing 50 $\mu g\,L^{-1}$ of correspondent antibiotic plus 20% (v/v) glycerol (Sigma–Aldrich Co.). Antibiotics were supplied by Sigma–Aldrich Co. and were added to the appropriate media at 50 $\mu g\,mL^{-1}$ of both ampicillin and kanamycin.

Plasmids pGFPuv, pAmCyan, pZsGreen, pZsYellow, pmOrange, ptdTomato, and pmCherry (Clontech Laboratories Inc.) were used as FP DNA source for cloning and expression. Plasmids pYes3/CT from Invitrogen and pQE-T7-2 (Qiagen), were used as host for the construction of transformed *E. coli* strains containing the gene templates of the selected recombinant FPs (Table S2).

DNA Isolation and Analysis. Plasmids from *E. coli* were isolated using the commercial Qiagen Plasmid Midiprep kit (Qiagen) according to the manufacturer's recommendations. The plasmids were subjected to electrophoresis on 0.8% (w/v) agarose precast gels stained with ethidium bromide (EB) using an E-Gel® 96-gel system (Invitrogen) and were visualized using UV transillumination. The purified DNA plasmids were quantified by fluorescence using a Quant-iT™ PicoGreen assay (Molecular Probes Inc.) following manufacturer's instructions.

Recombinant DNA Techniques. In Table S2 are depicted the source FPs vectors, oligonucleotide primers, plasmid constructs, and other specific conditions used to obtain specific genetic libraries. Recombinant DNA techniques were carried out following conventional protocols described previously by Sambrook and Russell (1). Briefly, the reaction polymerase chain reaction (PCR) mixtures contained 5 μL of each primer (10 μM) (Integrated DNA Technologies), 10 μL of 5× Phusion High Fidelity (HF) buffer, 1 μL of dNPs mix (10 mM of each) and 0.5 μL of Phusion® Hot Start II DNA High Fidelity DNA polymerase from New England Biolabs. The reaction pool was completed by the addition of 1 μL DNA template (10 ng) and the total volume was adjusted to 50 μL with nuclease free water (Sigma–Aldrich Co.).

The PCR were performed in a MWG-Biotech Primus 96 Plus Thermal Cycler (Lab. Extreme Inc.). The reaction started with 30 s of denaturation at 98 °C followed by 25 cycles of denaturation at 98 °C for 7 s, 30 s annealing at the primers specific temperature (Table S2) and 30 s at 72 °C. An additional 10 s for the extension completed the reaction. A negative control sample, where no template DNA was used, and a positive control sample containing purified λ DNA (Invitrogen), were included in all the PCR batches. Chimeric DNA (cDNA) recombinant FP amplicons were purified with a Qiaquick purification kit for purification of 100 bp to 10 kb PCR products (Qiagen).

Construction of the Plasmids pYES3/CT FPs. Both *Kpn*I and *Eco*RI amplified FP cDNA fragments (GFPuv, AmCyan, ZsGreen, ZsYellow, mOrange, tdTomato, and mCherry) were double digested with *Kpn*I High Fidelity (HF)/*Eco*RI HF enzymes (New England Biolabs) to produce 5′-base and 3′-base overhangs compatible with the selected host plasmid pYes3/CT (Table S2). After purification and quantification (see section above), the inserts were ligated with T4 DNA Ligase (Quick Ligation kit, New England Biolabs) into the pYes2 or pYes3/CT plasmids digested with *Kpn*I HF and *Eco*RI HF to give rise to the pYes3/CT (FP) plasmid libraries such as pYes3/CT (GFPuv), pYes3/CT (AmCyan), pYes3/CT (ZsGreen), pYes3/CT (ZsYellow), pYes3/CT (mOrange), pYes3/CT (tdTomato), pYes3/CT (mCherry) or pYes2 (GFPuv), pYes2 (AmCyan), pYes2 (ZsGreen), pYes2 (ZsYellow), pYes2 (mOrange), and pYes2 (mCherry).

For all library construction methods, both chemically competent *E. coli* strains, One Shot® TOP10 and BL21 Star™ (DE3) pLysS One Shot®, were transformed and grown on LBA and TB as described above. Colonies of interest were manually screened as previously described (2) and single colonies were inoculated in TB medium and incubated at 37 °C for 8 h. Finally, bacterial cells were harvested by centrifugation at 6,000 × *g* for 15 min at 4 °C and the pellets were treated to extract the plasmids as detailed above.

Construction of the Plasmids pQE-T7-2 (FP)s. The general procedures were essentially similar to those described in the previous sections. Briefly, FP cDNA fragments (GFPuv, AmCyan, ZsGreen, ZsYellow, mOrange, tdTomato, and mCherry) were amplified from source vectors (see Table S2) using primers, which incorporate the *Sac*I and *Kpn*I restriction sites into 5′- and 3′- termini. The double digested cDNA fragments were purified, quantified and ligated to the plasmid host pQE-T7-2 that had been digested with the restriction enzymes, *Sac*I HF and *Kpn*I HF (New England Biolabs). The final recombinant plasmids, pQE-T7-2 (GFPuv), pQE-T7-2 (AmCyan), pQE-T7-2 (ZsGreen), pQE-T7-2 (ZsYellow), pQE-T7-2 (mOrange), pQE-T7-2 (tdTomato). and pQE-T7-2 (mCherry), were retransformed into both chemically competent *E. coli* strains, One Shot® TOP10 and BL21 Star™ (DE3) pLysS One Shot® under the conditions specified above.

Fluorescent Proteins Spectra. The fluorescence emission spectra were recorded using a Varian (Cary Eclipse™) Fluorescence Spectro-photometer. The wavelength corrersponding to the maximum excitationwas used to record the emission spectrum for each FP (Fig. S2).

Preparation of Steganography by Printed Arrays of Microbes (SPAMs). To produce the colony matrices, single cell colonies of fluorescent bacterial strains were grown in their corresponding selection media, washed with 1× PBS to eliminate any residual antibiotic, resuspended in Terrific Broth and transferred to a source microtiter plate. A 96-pin Multi-Blot™ replicator (V&P scientific Colony Copier™ VP 409) was used to inoculate the arrays of colonies on LB agar casted on an Omni Tray (Nunc), with the appropriate antibiotics. The SPAMs were then incubated accordingly. For the IPTG induction experiments, the arrays of colonies were prepared following the same procedure. After 18 h of incubation, a 10 mM solution of IPTG (Sigma) was sprayed onto the colony arrays and the plates were placed back in the incubator.

Image Acquisition and Analysis. In the preliminary studies the images were acquired using a color camera (Nikon D7000 equipped with a Nikkor lens 18–200 mm, F/3.5–5.6) in a epi-illumination setup. Light-emitting diodes (LEDs) from ThorLabs were used as excitation sources to determine the optimal excitation light source and emission filters. The emission filters used in this work are Wratten Gel Filter (Kodak) and the exact cut-offs were determined by absorption spectroscopy as indicated in Fig. S2, *Right*. Fig. S3, *Left* shows several possible outcomes for a combination of excitation and emission wavelength when imaged with a color camera.

After determining that the combination of $\lambda_{exc} = 470$ nm and $\lambda_{em} > 535$ nm shows discernibles signal from all seven FPs, we used a Safe Imager™ 2.0 Blue Light Transilluminator (Invitrogen) equipped with an array of blue LEDs (approximately 470 nm) and an amber filter unit, which cut-off is shown in Fig. S2, *Right* (InvFilter). The images of the SPAM were acquired using a digital single-lens reflex (DSLR) color camera (Nikon D7000 equipped with a Nikkor lens 18–200 mm, F/3.5–5.6) or alternatively the camera of a smartphone (Apple iPhone 4). Fig. S4 shows the comparison between the images acquired with both detection systems.

1. Sambrook J, Russell DW (2001) *Molecular Cloning: A Laboratory Manual*, 3rd Ed (Cold Spring Harbor, New York).
2. Baird GS, Zacharias DA, Tsien RY (1999) Biochemistry, mutagenesis, and oligomerization of DsRed, a red fluorescent protein from coral. *Proc Natl Acad Sci USA* 96:11241–11246.

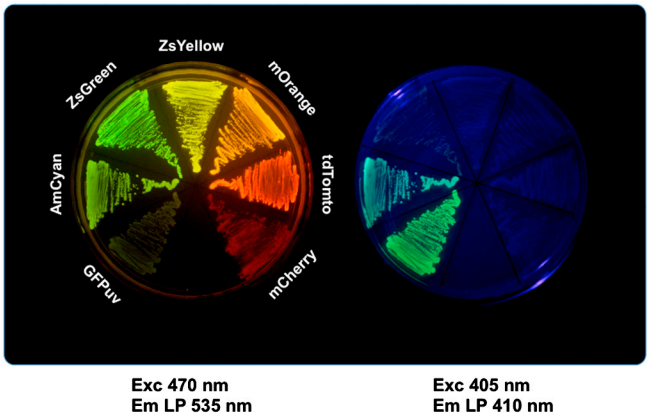

Fig. S1. Fluorescence image of streaks of TOP10 *E.coli* cells transformed with seven different fluorescent proteins [pYES3/CT (FP)] after growing for 36 h at 37 °C. The same plate was imaged with two different excitation light sources through different emission filters.

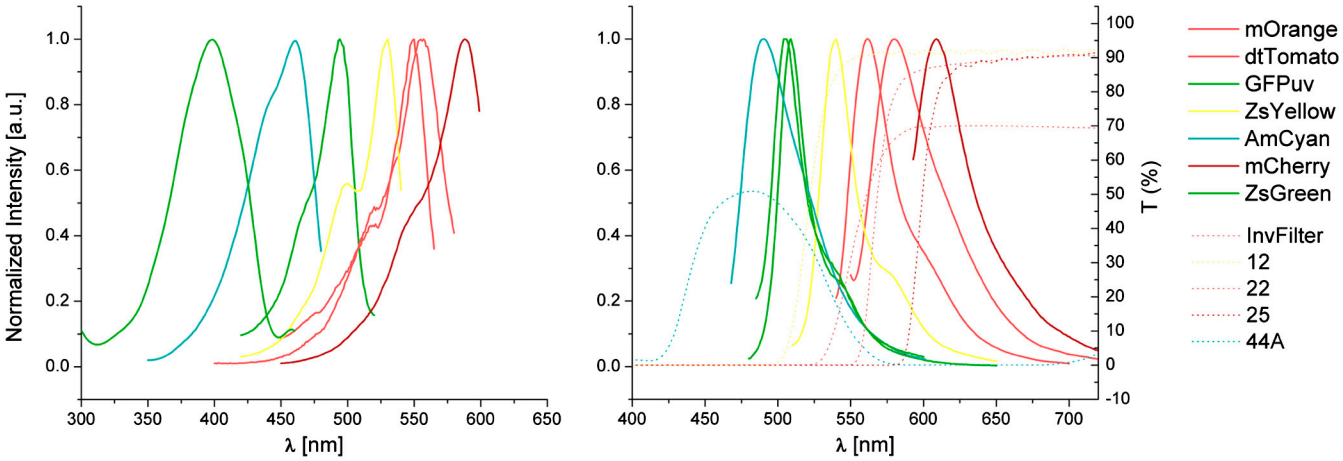

Fig. S2. Normalized excitation and emission spectra overlaid with the different emission filters used in this study.

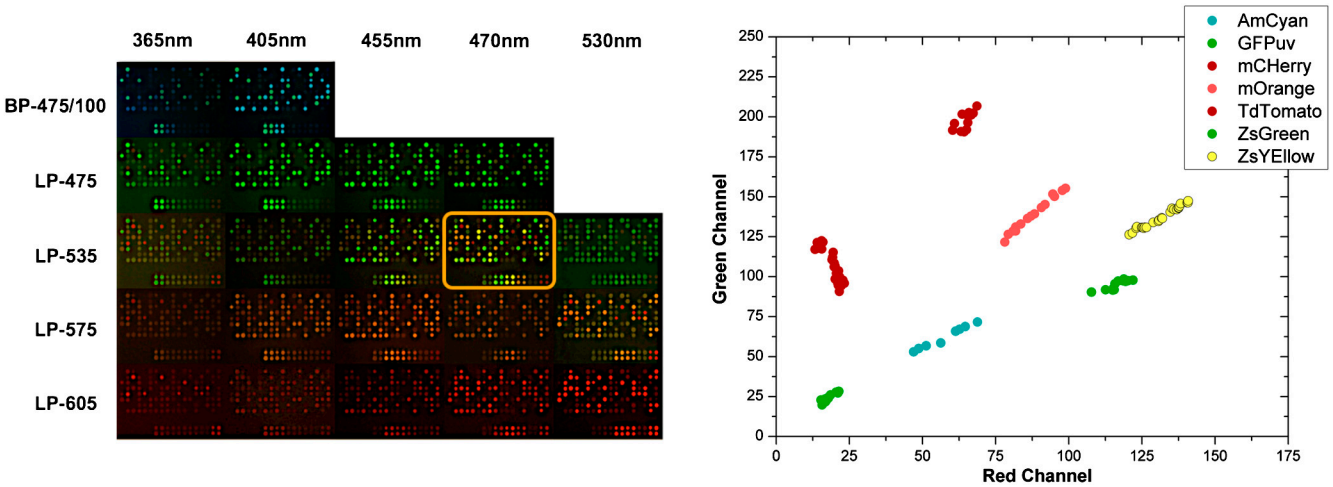

Fig. S3. (*Left*) Fluorescence images of a SPAM captured with a color camera using different combinations of excitation and emission wavelength. (*Right*) Scatter plot of the green and red channel components from the image of the SPAM ($\lambda_{exc} = 470$ nm; $\lambda_{em} > 535$ nm). The blue channel response is negligible because the blue emission is optically filtered. The plot shows seven different clusters of signal corresponding to seven different FPs contained in the SPAM.

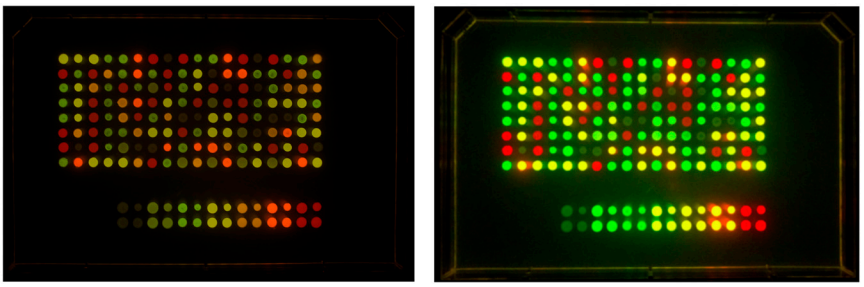

Fig. S4. Fluorescence images of BL21(DE3)pLysE fluorescent strains after growth and induced FP expression by IPTG. Left photograph taken with a Nikon D7000 and right photograph taken with an iPhone 4.

Table S1. Bacterial strains used in this work

E. coli strain	Genotype	Source
One Shot® TOP10	F- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*araleu*) 7697 *gal*U *gal*K *rps*L (StrR) *end*A1 *nup*G	Invitrogen
BL21 Star™ (DE3) pLysS One Shot®	F- *ompT hsdS*B (rB-mB-) *gal dcm rne131* DE3)* LysS (CamR)	Invitrogen

*DE3 indicates the strains contain the DE3 lysogen that carries the gene for T7 RNA polymerase under control of the *lac*UV5 promoter. IPTG is required to induce expression of the T7 RNA polymerase.

Table S2. Constructed bacterial strains, plasmids, and primers libraries used in this work

Constructed *E. coli* strain	Host expression plasmid	Source FP DNA plasmid	Primers 5′ → 3′	Annealing T, °C	Restriction enzymes	Observations
Yes3/CT (GFPuv) IYes3/CT (GFPuv) II	pYes3/CT	pGFPuv	[a,b]Forward Primer (FP): GCGGTACCTGGAAAAATGTCTAGTAAAGGAGAAGAACTTTTCACT Reverse Primer (RP): CGGAATTCTTATTTGTAGAGCTCATCCATGCC	61		
Yes3/CT (AmCyan) I Yes3/CT (AmCyan) II		pAmCyan	FP: GCGGTACCTGGAAAAATGTCTGCTCTTTCAAACAAGTTTATCGGA RP: CGGAATTCTCAGAAAGGGACAACAGAGGT	66		
Yes3/CT (ZsGreen) IYes3/CT (ZsGreen) II		pZsGreen	FP: GCGGTACCTGGAAAAATGTCTGCTCAGTCAAAGCACGGTCTA RP: CGGAATTC	68		
Yes3/CT (ZsYellow) IYes3/CT (ZsYellow) II		pZsYellow	FP: GCGGTACCTGGAAAAATGTCTGCTCAGTCAAAGCACGGTCTA RP: CGGAATTC	68	*Kpn*I HF *Eco*RI HF	
Yes3/CT (mOrange) IYes3/CT (mOrange) II		pmOrange	FP: GCGGTACCTGGAAAAATGTCTGTGAGCAAGGGCGAGGAGAAT RP: CGGAATTCCTTGTACAGCTCGTCCATGCC	62		
Yes3/CT (tdTomato) IYes3/CT (tdTomato) II		ptdTomato	FP: GCGGTACCTGGAAAAATGTCTGTGAGCAAGGGCGAGGAG RP: CGGAATTCCTTGTACAGCTCGTCCATGCCGTA	62		FP: Kozac Consensus RP: STOP codon [a]Underlined bases indicate restriction sites; [b]Bold base indicate reading frame correction [c]I and II corresponding to both stream sources used
Yes3/CT (mCherry) IYes3/CT (mCherry) II		pmCherry	FP: GCGGTACCTGGAAAAATGTCTGTGAGCAAGGGCGAGGAGGAT RP: CGGAATTCCTTGTACAGCTCGTCCATGCC	62		
QE-T7-2 (GFPuv) I[c] QE-T7-2 (GFPuv) II	pQE-T7-2	pGFPuv	[a,b]Forward Primer (FP): GCGAGCTCTGGAAAAATGTCTAGTAAAGGAGAAGAACTTTTCACT Reverse Primer (RP): CGGGTACCTTATTTGTAGAGCTCATCCATGCC	61		
QE-T7-2 (AmCyan) I, QE-T7-2 (AmCyan) II		pAmCyan	FP: GCGAGCTCTGGAAAAATGTCTGCTCTTTCAAACAAGTTTATCGGA RP: CGGGTACCTCAGAAAGGGACAACAGAGGT	66		
QE-T7-2 (ZsGreen) IQE-T7-2 (ZsGreen) I		pZsGreen	FP: GCGAGCTCTGGAAAAATGTCTGCTCAGTCAAAGCACGGTCTA RP: CGGGTACCGGCCAAGGCAGAAGGGAATGC	68		
QE-T7-2 (ZsYellow) IQE-T7-2 (ZsYellow) II		pZsYellow	FP: GCGAGCTCTGGAAAAATGTCTGCTCAGTCAAAGCACGGTCTA RP: CGGGTACCGGCCAAGGCAGAAGGGAATGC	68	*Sac*I HF*Kpn*I HF	
QE-T7-2 (mOrange) IQE-T7-2 (mOrange) II		pmOrange	FP: GCGAGCTCTGGAAAAATGTCTGTGAGCAAGGGCGAGGAGAAT RP: CGGGTACCCTTGTACAGCTCGTCCATGCC	62		
QE-T7-2 (tdTomato) IQE-T7-2 (tdTomato) II		ptdTomato	FP: GCGAGCTCTGGAAAAATGTCTGTGAGCAAGGGCGAGGAG RP: CGGGTACCCTTGTACAGCTCGTCCATGCCGTA	62		
QE-T7-2 (mCherry) IQE-T7-2 (mCherry) II		pmCherry	FP: GCGAGCTCTGGAAAAATGTCTGTGAGCAAGGGCGAGGAGGAT RP: CGGGTACCCTTGTACAGCTCGTCCATGCC	62		