A synthetic three-color reporter framework for monitoring genetic regulation and noise

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Abstract

Biologists require accurate, distinguishable, non-toxic reporters for multiple genes in the same organism. Despite recent improvements in fluorescent proteins, there does not exist a single vector with which one can conveniently employ multiple reporters. Therefore, we designed and built such a system using total DNA synthesis. This framework will be useful for analyzing natural genetic circuits--as well as assembling synthetic circuits--in many organisms. Here we characterize the framework in *Escherichia coli*. Three spectrally distinct reporters allow independent monitoring of genetic signals and analysis of genetic noise. As an application, we show that the framework is a sensitive detector of transcriptional co-regulation.

Extended Abstract

We designed a three-color fluorescent reporter framework (Fig. 1) to fufill several criteria: (1) *Stability and biocompatability*. The framework is genetically stable and non-toxic to cells carrying it. This was accomplished by keeping the framework small (4kb), using a low copy plasmid origin of replication (SC101), moderate strength ribosome binding sites (RBSs), optimizing the fluorescent proteins to remove "toxic" codons, and placing them under the control of tightly regulated promoters[1]. To discourage mutation, we explicitly avoided homologous or repeated sequences. (2) *Brightness, tightness, and distinctness.* We chose fast maturing, monomeric proteins with minimally overlapping spectra to maximize linearity of response[2], along with initial promoters that could be tightly regulated[1]. (3) *Sensitivity and independence.* We wished to detect both strong and weak genetic signals simultaneously, with the ability to watch them change over time in single living cells[3]. We used multiple genetic terminator sequences[4] along with empty "spacer" regions to insure that changing the expression level of one protein did not affect the level of another, except when genetically co-regulated (Fig. 2). (4) *Tunability.* The framework design allows for easy tuning of reporter parameters: promoter strengths, RBSs, and degradation tags, as well as the construction of fluorescent fusions. (5) *Modularity, portability, and extensibility.* Restriction sites were strategically placed to allow genetic elements to be easily swapped, inserted, or deleted. The fluorescent proteins were codon-optimized for both gram positive and gram negative bacteria. The system can be moved between different plasmids[1], chromosomes, and organisms. Additional restriction sites are provided to add a fourth reporter and promoter.

We characterized the framework in single *E. coli* cells using quantitative fluorescence microscopy (Fig. 2). In our strain, all three fluorescent proteins are repressed. Cells carrying the plasmid showed very weak (5%), but detectable, expression of the *yfp* and *cfp* genes compared to the mean cellular autofluorescence (Fig. 2, columns 0 and I). We tested whether individual operons in the framework plasmid could be induced independently. We found no transcriptional read-through from *rfp* into *cfp* (Fig. 2, columns V and VII). Similarly, the mean expression of *cfp* and *yfp* was independent (Fig. 2, columns I-IV). The combinatorial LacI/AraC regulated promoter[1] controlling *rfp* behaved as an asymmetric-AND gate (Fig. 2, columns I, III, V, and VII). Finally, we found that the correlation of genetic noise between *rfp* and *yfp* sensitively measured their co-regulation by LacI, in both steady-state and time-lapse experiments (not shown).

We can now design extremely complex genetic sequences with well-controlled behavior, by exploiting total DNA synthesis. This quantitative reporter system will allow unprecedented quantitative analysis of natural and synthetic gene networks. Simultaneously, this system will test and expand our DNA sequence-level knowledge of the regulation of gene expression.

Figure 1. (A) The framework. Terminators are hatched boxes. RBSs are purple circles. Restriction sites are blue bars. The promoters are small black arrows. Each fluorescent operon is shown as a colored block arrow, while the (ampicillin) antibiotic resistance is shown as a black block arrow. (B) The framework is measured in the low-copy SC101 plasmid in the MG1655 strain[5] containing the native *ara* operon, and the LacI and TetR over-expressing Z1 cassette[1]. The three reporters are controlled by promoters[1] responsive to: tetracycline/aTc (*cfp*), lactose/IPTG (*yfp*), and both lactose/IPTG and arabinose/Lara (*rfp*).

Figure 1.

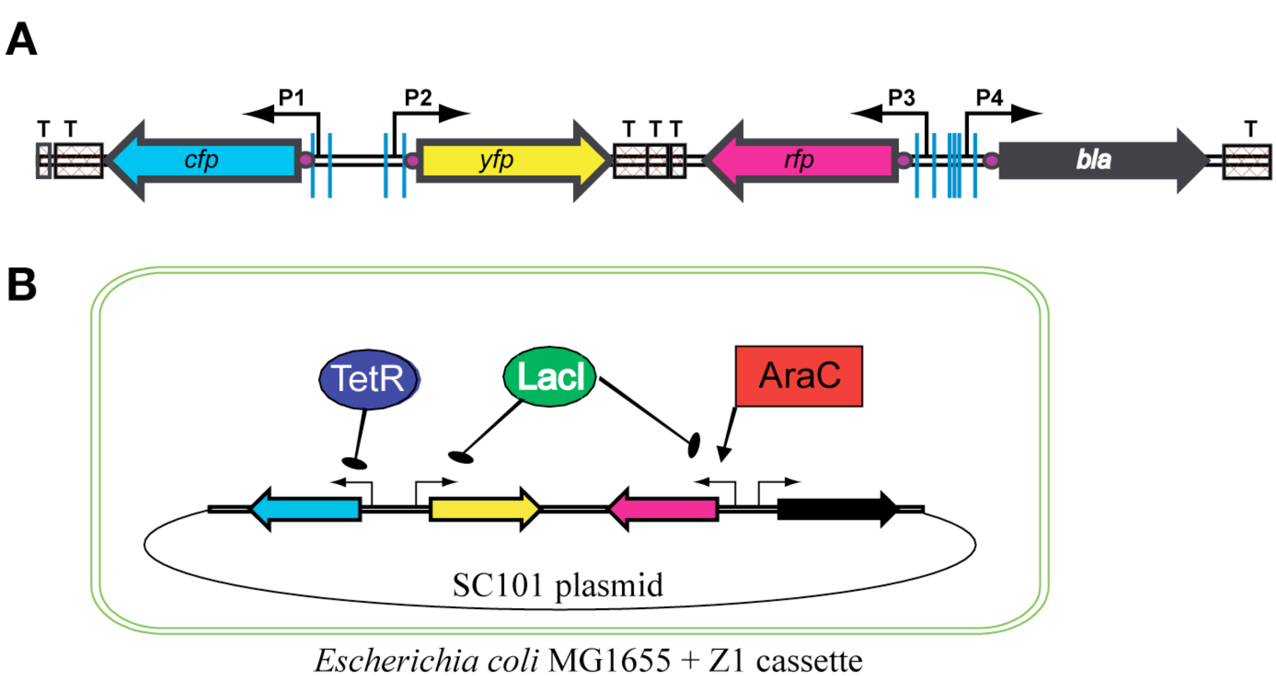
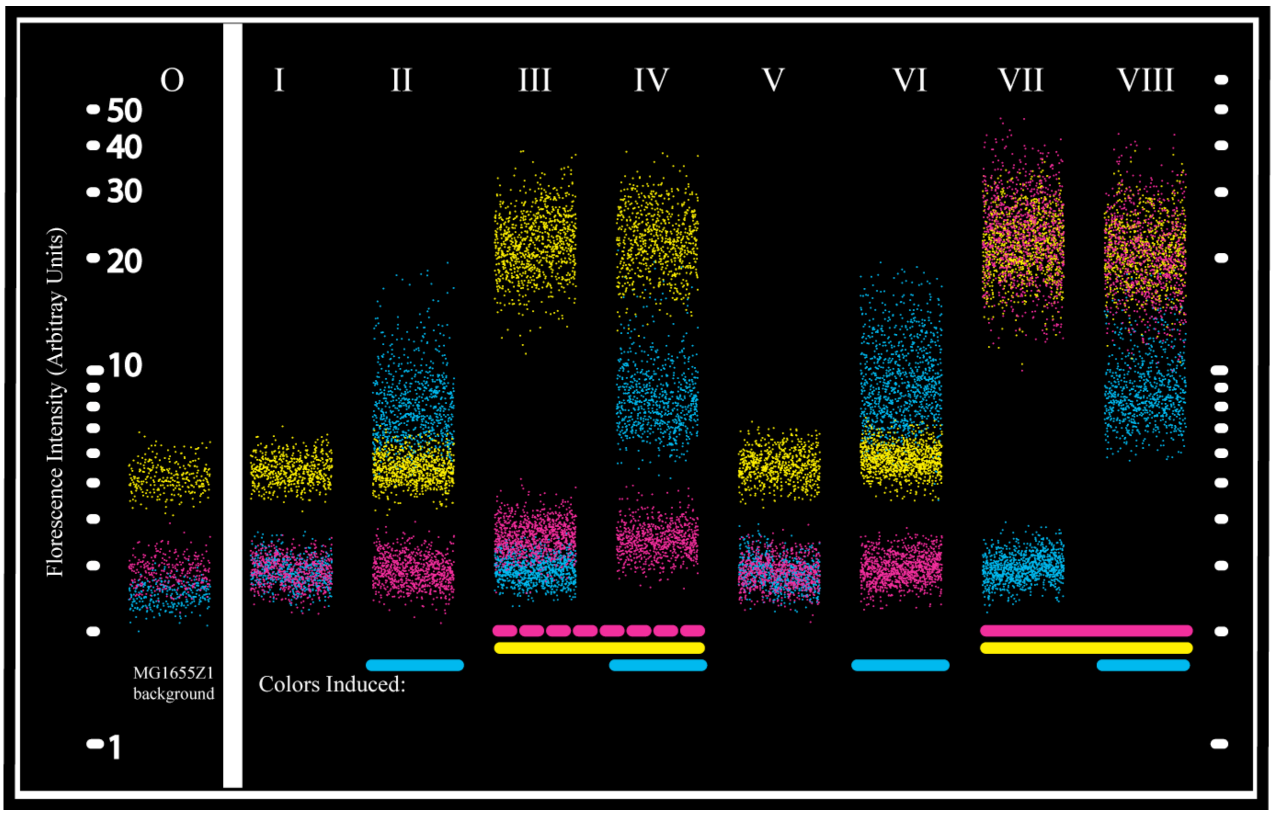


Figure 2. Operons allow independent control of gene expression. Fluorescence microscopy snapshots were taken under each combination of inducer conditions. This plot shows the response of each reporter to different combinations of these three inducers (each column is one condition, the color expected is shown as a bar below). Each cell within the population is represented by three dots--one of each color--in order to show the genetic noise in each condition. Note that the LacI/AraC promoter (*rfp*) operates as an 'asym-AND' gate: its expression is only slightly increased (3%) by IPTG alone, and not (0%) by Lara alone.



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