



**BGSC Accession:** ECE180 (pICFP), ECE181 (pIYFP)

**Original Code:** TG90(pICFP) and TG90(pIYFP), respectively

**Reference:** Veening, J-W. (manuscript submitted).

**Sequence:** Available at [www.bgsc.org/sequences/pICFP.htm](http://www.bgsc.org/sequences/pICFP.htm) and [www.bgsc.org/sequences/pIYFP.htm](http://www.bgsc.org/sequences/pIYFP.htm)

**Features:**

- icpF*, *iyfF* encode fluorescent protein variants (cyan and yellow, respectively) engineered for improved expression and therefore greater sensitivity in *B. subtilis*.
- cat* encodes chloramphenicol acetyl transferase; selectable in either *E. coli* or *B. subtilis* (chloramphenicol 5 µg/ml)
- bla* encodes β-lactamase; selectable in *E. coli* only (ampicillin 100 µg/ml)
- ori-pUC*, *ori-f1* plasmid replication origins, functional in *E. coli* but not Gram-positive bacteria

**Description:** Integration vector; allow creation of fluorescent protein fusions for the study of gene expression

**Construction:** To improve expression of GFP variants in *B. subtilis*, several codons were added to the 5' end of the *cfp* or *yfp* coding sequence.

**Use:** The plasmids are designed to create a fusion into either of two GFP variants, ICFP and IYFP, modified for higher expression of Cyan and Yellow Fluorescent Proteins, respectively, in *B. subtilis* 168. A *B. subtilis* gene is inserted into the multiple cloning site just upstream from the *icfP* or *iyfP* genes. The plasmid is then isolated from the *E. coli* host and used to transform the recipient *B. subtilis* strain, with selection for chloramphenicol resistance. Integrants should have the fluorescent protein fusion placed under the controls operative at the *B. subtilis* locus.

**Recipient strains:** pICFP and pIYFP work well with recipients derived from *B. subtilis* 168, but should prove useful in a wide variety of Gram-positive species.

**Protocols:** *B. subtilis* competent cell preparation and transformation.