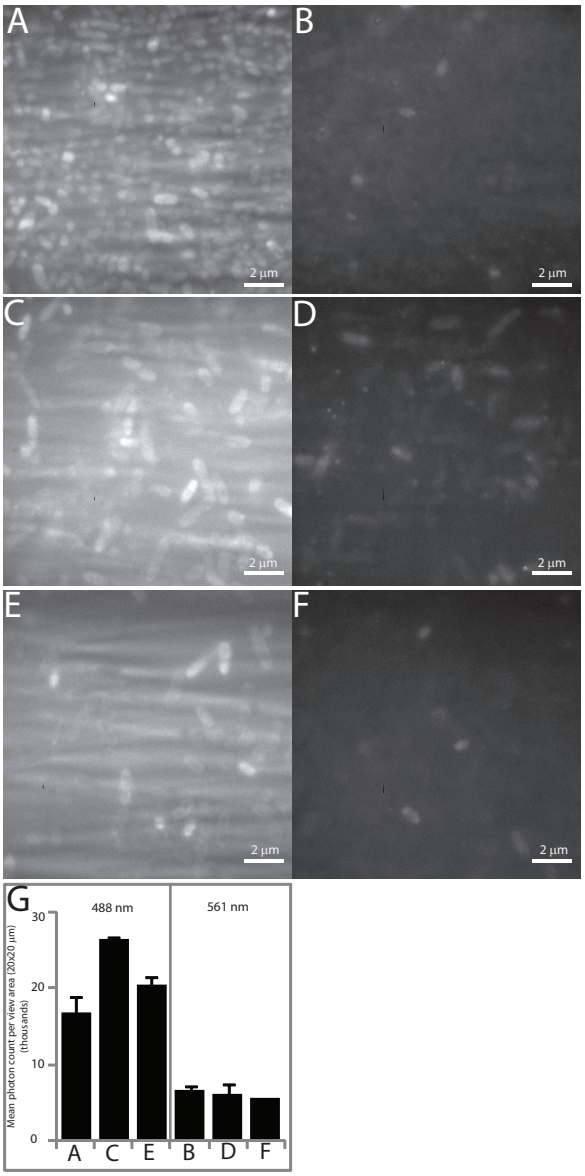

Figure S1. TIRF microscopy of engineered *E. coli* strains.
E. coli were affixed to a gelatin coated chambered glass coverslip and viewed after being illuminated with a 488 nm laser (left column) or a 561 nm laser (right column). **A**. **and B.** *E. coli* DH10B. **C. and D.** *E. coli* + pLY02 (lycopene-expressing). **E. and F.** *E. coli* + pTAR (actinorhodopsin-expressing, but without the retinalco-factor). **G.** Fluorescence intensity observed for each sample illuminated with the 488 nm laser or 561 nm laser. Bar labels correspond to the panels above.

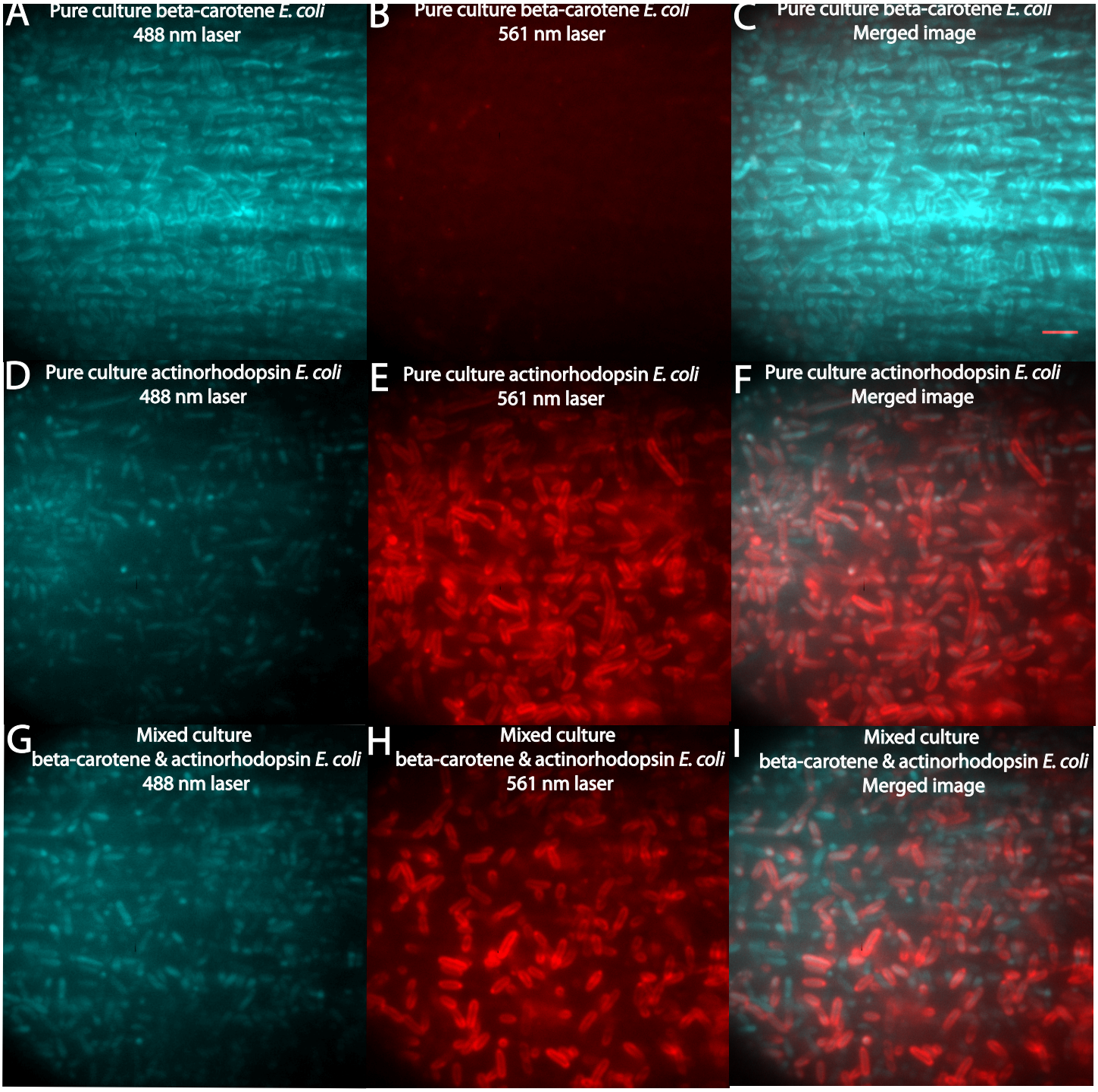

Figure S2: TIRF microscopy of pure cultures and mixed beta-carotene- and actinorhodopsin-expressing *E. coli*. A glass coverslip was coated with 0.5% gelatin, and cells were allowed to attach for 10 minutes. Unattached cells were washed away and the coverslip was flooded with media before imaging. The same field of view was excited with the 488 nm laser and the 561 nm laser. Fluorescence observed from the 488 nm laser was colored cyan (left panels), while fluorescence from the 561 nm laser was colored red (center panels), and the images were merged (right panels). **A**. A pure culture of *E. coli* with plasmid pBC01 (beta-carotene-expressing cells) was imaged with the 488 nm laser and fluorescence was colored cyan. **B**. The same field of view was imaged with the 561 nm laser, and fluorescence was colored red. **C**. The images from **A** and **B** were merged. **D**. A pure culture of *E. coli* with plasmids pRET04 and pTAR (actinorhodopsin-expressing cells) was imaged with the 488 nm laser and colored cyan. **E**. The same field of view was illuminated with the 561 nm laser and colored red. **F**. The images from **D** and **E** were merged. **G**. A mixed culture of beta-carotene- and actinorhodopsin-expressing cells was imaged with the 488 nm laser and colored cyan. Both beta-carotene- and actinorhodopsin-expressing cells are apparent here. **H**. The same field of view was illuminated with 561 nm laser. Only actinorhodopsin-expressing cells are visible. **I**. The images from **G** and **H** were merged showing the two populations of cells.

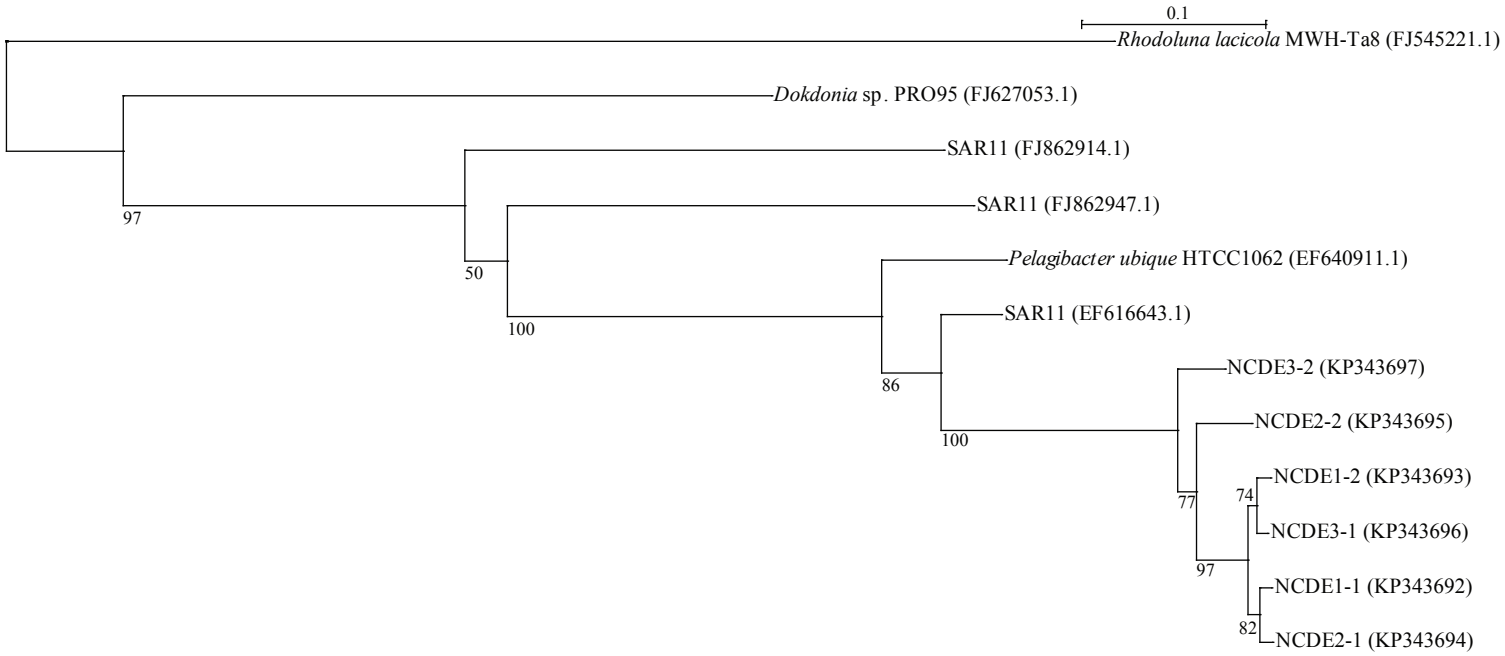

Figure S3: Phylogenetic tree of partial rhodopsin gene sequences.
Partial gene sequences recovered by PCR with degenerate SAR11-type rhodopsin primers were aligned with proteorhodopsin sequences from *Pelagibacter ubique*, *Dokdonia* sp., several SAR11-type uncultured clones, and the actinorhodopsin sequence from *Rhodoluna lacicola* MWH-Ta8. Several closely related sequences were obtained from the Delaware River water samples collected during this study. Accession numbers are provided in the parentheses.

Primer name	Primer sequence (5' →3')	Gene(s) in product	Template	Reference
LG1_F	(1) TAY MGN TAY GTN GAY TGG (2) MGN TAY ATH GAY TGG YT	*actR* (partial)	DE River genomic DNA	(1)
LG1_R	ATN GGR TAN ACN CCC CA	*actR* (partial)	DE River genomic DNA	(1)
LG2_F	TAY MGN TAY GCN GAY TGG	*actR* (partial)	DE River genomic DNA	(1)
LG2_R	ATN GGR TAN ACN CCC CA	*actR* (partial)	DE River genomic DNA	(1)
PCL1_F	(1) MGN TAY ATH GAY TGG YT (2) WWN MGN TAY GTN GAY TGG	*actR* (partial)	DE River genomic DNA	(1)
PCL1_R	(1) CCR AAN CCN ACY TTR TT (2) GGR TAD ATC ATC CAN CC	*actR* (partial)	DE River genomic DNA	(1)
PR-1aF	GAT CGA GCG NTA YRT HGA RTG G	*pR* (partial)	DE River genomic DNA	(2)
PR-1aR	GAT CGA GCR TAD ATN GCC CAN CC	*pR* (partial)	DE River genomic DNA	(2)
FlavoPRMI_459F	CCA GCA GGA GCA AAA ACA	*pR* (partial)	DE River genomic DNA	(2)
FlavoPRMI_555R	GCT ACC ACC TTC AGG ATT	*pR* (partial)	DE River genomic DNA	(2)

Table S1. Primers used for screening Delaware River water genomic DNA for rhodopsin genes. Degenerate primers for several different rhodopsin genes were used to screen genomic DNA isolated from the Delaware River. The primers listed in this table were unsuccessful at amplifying any rhodopsins in this study.

References

1. **Sharma AK, Sommerfeld K, Bullerjahn GS, Matteson AR, Wilhelm SW, Jezbera J, Brandt U, Doolittle WF, Hahn MW.** 2009. Actinorhodopsin genes discovered in diverse freshwater habitats and among cultivated freshwater Actinobacteria. Isme J **3:**726-737.

2. **Lami R, Cottrell MT, Campbell BJ, Kirchman DL.** 2009. Light-dependent growth and proteorhodopsin expression by Flavobacteria and SAR11 in experiments with Delaware coastal waters. Environ Microbiol **11:**3201-3209.