Supporting Information

Bussell and Kehoe 10.1073/pnas.1303371110

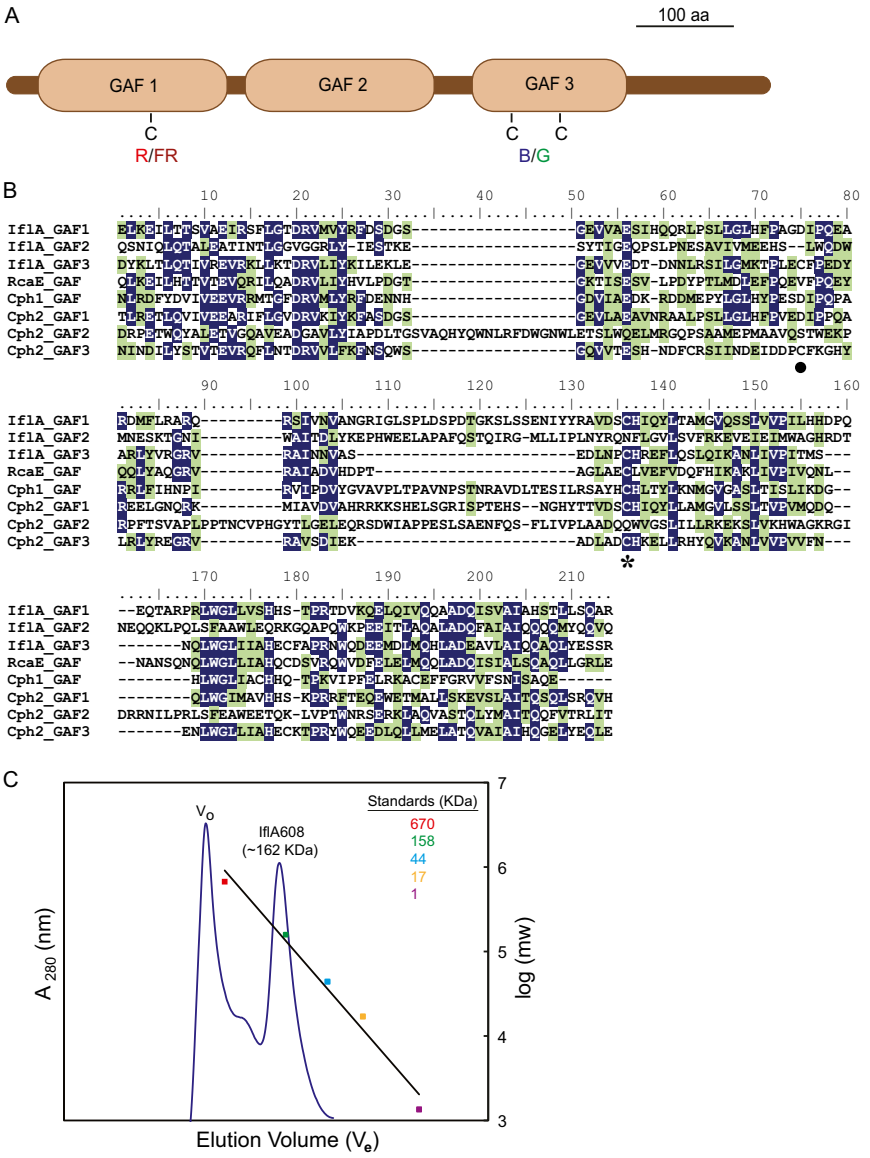

Fig. S1. IflA contains three GAF domains and is a dimer. (*A*) Schematic of IflA domain architecture, with the putative chromophore-binding cysteines in the red/far-red-responsive GAF1 and blue/green-responsive GAF3 domains. (*B*) Clustal W sequence alignments (1) of cyanobacterial phytochrome GAF domains. IflA and RcaE GAF domain sequences are from *Fremyella diplosiphon*, and Cph1 and Cph2 GAF domain sequences are from *Synechocystis* sp. PCC 6803. Blue shading denotes a minimum of 50% identical residues, and green shading represents a minimum of 50% similar residues at each position. The cysteine residue necessary for canonical phytochrome chromophore attachment is denoted by the star, whereas the cysteine required for chromophore attachment in the blue-green absorbing GAF domain subfamily is marked with a dot (2, 3). (*C*) IflA608 elution (peak indicated) from a size-exclusion column with various-sized standards provided. The experiment was conducted three times with similar results. Vo, void volume.

1. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22(22):4673–4680.
2. Rockwell NC, Martin SS, Gulevich AG, Lagarias JC (2012) Phycoviolobilin formation and spectral tuning in the DXCF cyanobacteriochrome subfamily. *Biochemistry* 51(7):1449–1463.
3. Ulijasz AT, et al. (2009) Cyanochromes are blue/green light photoreversible photoreceptors defined by a stable double cysteine linkage to a phycoviolobilin-type chromophore. *J Biol Chem* 284(43):29757–29772.

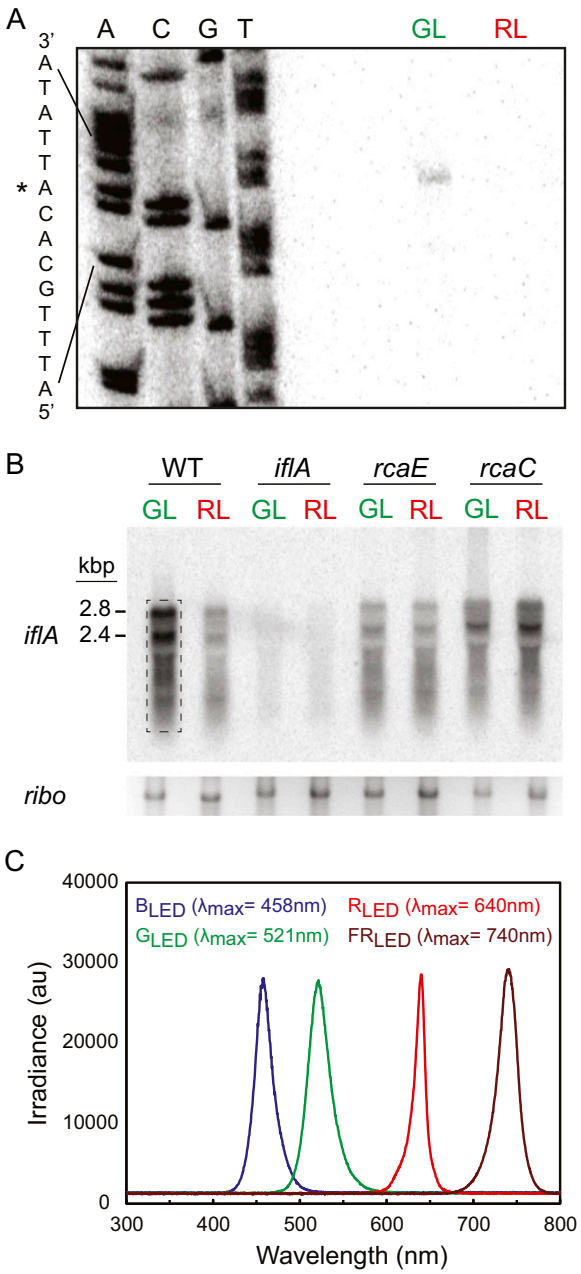

Fig. S2. Transcriptional start site of *iflA* is located within 5′-end of the L box sequence and produces two transcripts. (*A*) Primer extension was used to determine the *iflA* transcription start site during growth in green light (GL) and red light (RL). The DNA sequencing ladder and the sequence and start site (denoted by an asterisk) are shown to the left. The result shown was obtained in three independent experiments. (*B*) Representative RNA blot autoradiograph for *iflA* in wild-type and *iflA*, *rcaE*, and *rcaC* null mutants. Dashed box, region measured for histogram values. *ribo*, 16S ribosomal bands. (*C*) Spectral distribution and emission maxima of the light provided by the LED sources used in this work.

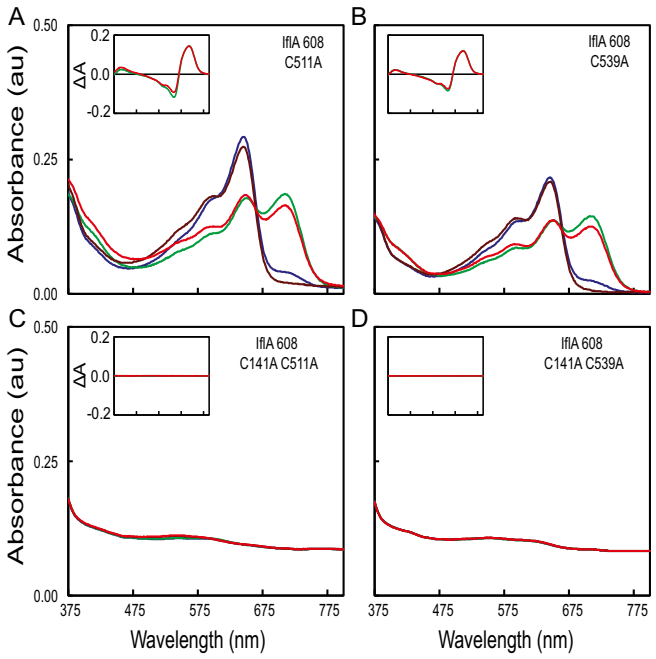

Fig. S3. Effects of cysteine to alanine substitutions on IflA608 absorption spectra. (*A–D*) Absorption spectra of purified IflA608 cysteine mutants. Line colors indicate light treatment provided before scanning. Difference spectra for green minus blue (green line) and red minus far red (red line) are shown (*Insets*).

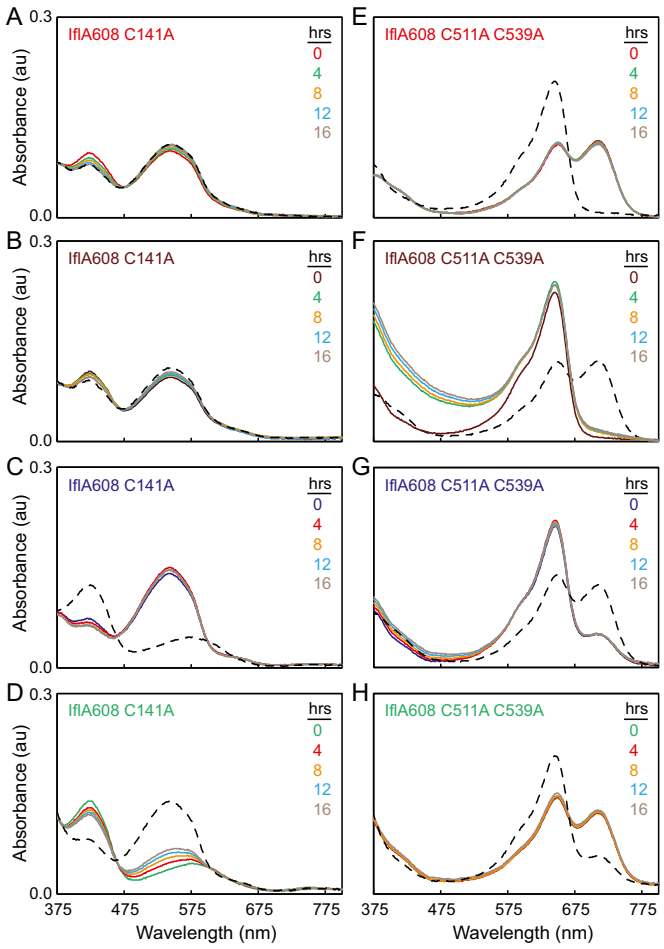

Fig. S4. The red-absorbing form of the IflA GAF1 domain does not accumulate during dark treatment when GAF3 is not chromophorylated. Sixteen-hour dark reversion analysis of IflA608-C141 after red (*A*), far-red (*B*), blue (*C*), or green (*D*) light exposure and of IflA608-C511A/C539A after red (*E*), far-red (*F*), blue (*G*), or green (*H*) light exposure. For all, line color denotes length of time in the dark after the initial light treatment (*Insets*), and black dashed lines are spectra after irradiation with opposite light color (red/far-red or blue/green) postdark treatment.

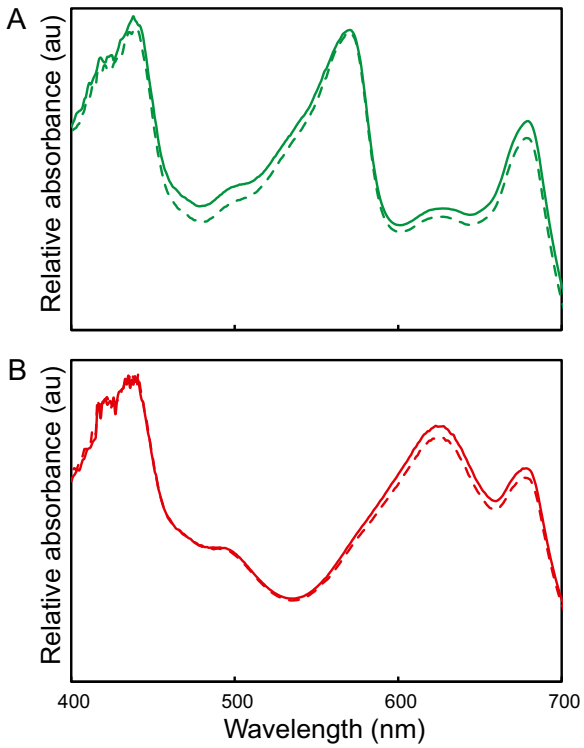

Fig. S5. Photosynthetic light harvesting pigment to chlorophyll ratios and CA3 in wild-type and *ifIA* cells are equivalent in green and red light. Wild-type (solid line) and *ifIA* (dashed lines) cells were grown in (*A*) green light and (*B*) red light. Relative absorbance was measured for cells after 7 d of growth in 20-μmol photons $m^{-2}{\cdot}s^{-1}$ light. Chlorophyll peaks are present at 430 and 680 nm, phycoerythrin (light-harvesting protein found in green light) peak is at 565 nm, and phycocyanin (light-harvesting protein found in red light) peak is at 620 nm. Experiments were carried out a minimum of three times using three independent isolates of wild-type and *ifIA* cells. Results comparable to those shown were obtained in all cases.

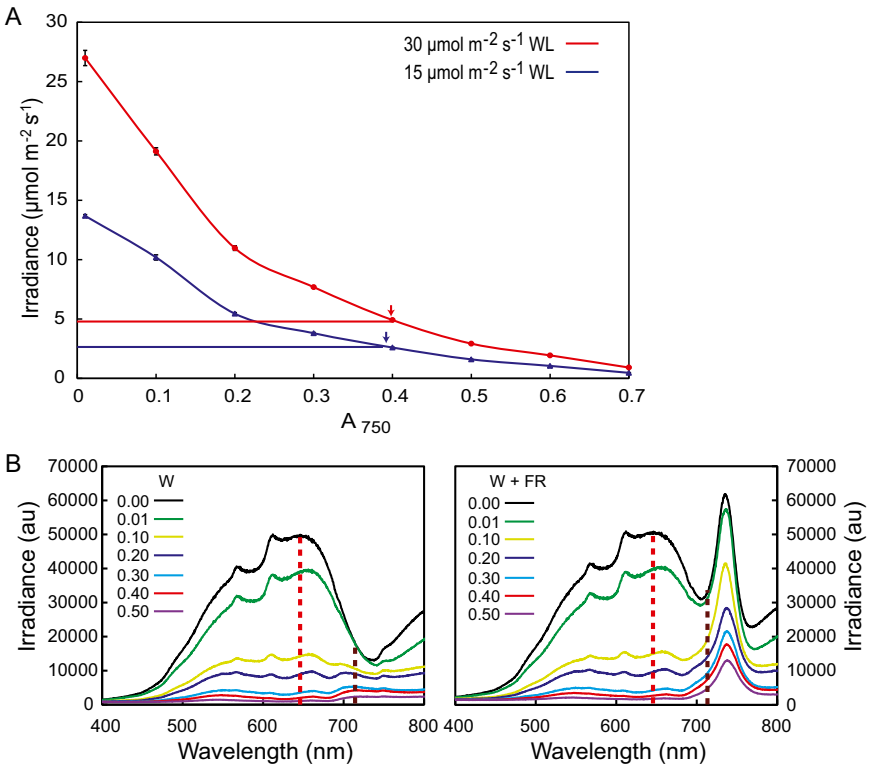

Fig. S6. Increasing culture density strongly affects light transmittance and wavelength distribution. (*A*) The amounts of light transmitted through the shaded sides of culture tubes that were laterally, unidirectionally illuminated and contained *F. diplosiphon* cultures of varying densities were measured for two different irradiance levels of white light (WL). The amounts of light transmitted through the tubes at an absorbance at 750 nm of ~0.4 (arrows), the density above which the growth of *iflA* cells was comparable to wild-type cells in both 30- and 15-µmol photons $m^{-2}{\cdot}s^{-1}$, are denoted by the horizontal red and blue lines. (*B*) Spectral distribution of light transmitted through culture tubes as described in *A* when illuminated with 15-µmol photons $m^{-2}{\cdot}s^{-1}$ white light (*Left*, W) or the same white light with 5-µmol photons $m^{-2}{\cdot}s^{-1}$ of far-red light added (*Right*, W+FR). Values under W and W+FR represent absorbance at 750 nm readings of cultures before each light measurement. Dotted red and far-red lines in both graphs show the levels of red and far-red light, respectively, transmitted through culture tubes containing BG-11 medium only in the two light conditions.

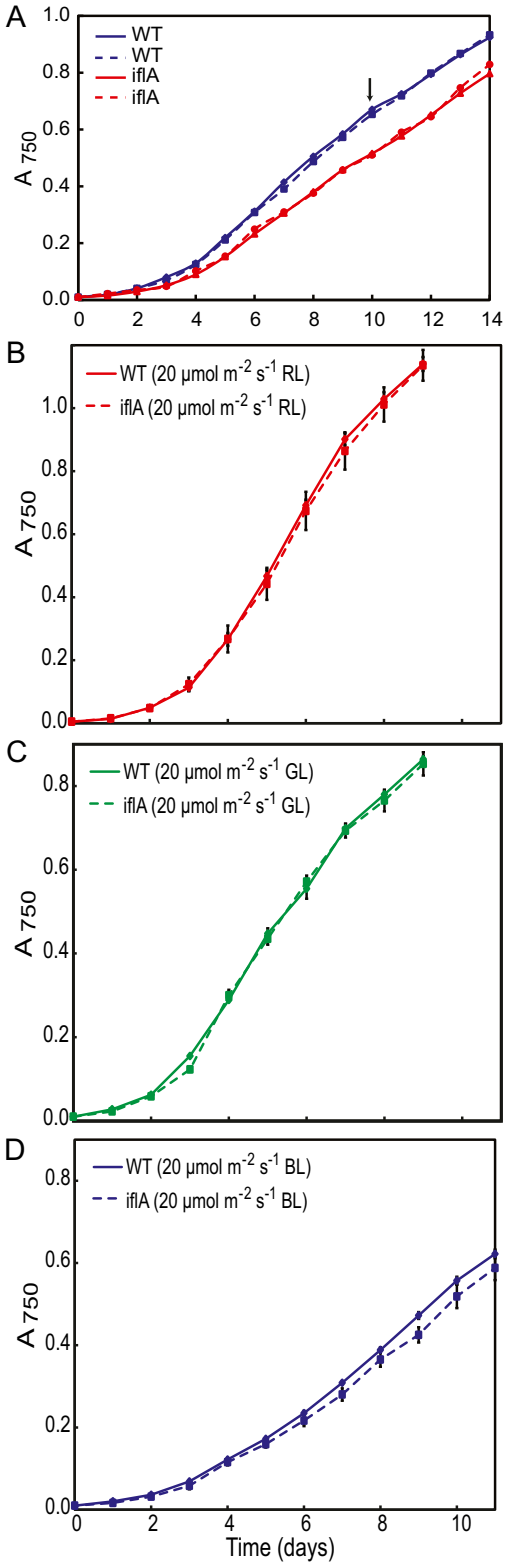

Fig. S7. Growth of wild-type and *iflA* cells in different media and light color conditions. (*A*) Wild-type (WT) and *iflA* cells were grown either without any media replacements (solid lines) or with media replaced (dashed lines) on the tenth day (arrow). Wild-type and *iflA* cells were grown in (*B*) red light (RL), (*C*) green light (GL), or (*D*) blue light (BL). Experiments were carried out a minimum of three times. Error bars denote SEM.

Table S1. Absorption maxima of IflA regions isolated from photoconvertible phycocyanobilin-producing *Escherichia coli* (in nm)

IflA Region	Pb λ_{max}	Pg λ_{max}	Pr λ_{max}	Pfr λ_{max}
IflA GAF 1	—	—	645	688
IflA GAF 3	421	545	—	—
IflA 608	415	545	645	708
IflA 608 C141A	415	545	—	—
IflA 608 C511A	—	—	645	708
IflA 608 C539A	—	—	645	708
IflA 608 C141A C511A	—	—	—	—
IflA 608 C141A C539A	—	—	—	—
IflA 608 C511A C539A	—	—	645	708
IflA 608 C141A C511A C539A	—	—	—	—

Table S2. Transmitted red/far-red light ratio variation with cell culture density

	White light		White light + far-red	
Absorbance at 750 nm	R/FR	FR/R	R/FR	FR/R
0.00	2.34	0.43	1.60	0.63
0.01	1.90	0.53	1.33	0.75
0.10	1.28	0.78	0.86	1.16
0.20	0.95	1.06	0.74	1.35
0.30	0.65	1.53	0.51	1.96
0.40*	0.49	2.03	0.42	2.39
0.50	0.47	2.11	0.43	2.32

*Absorbance at 750 nm of cultures above which *iflA* mutants resemble wild-type cell growth. FR, far-red; R, red.

Table S3. Primer sequences and expression plasmids used in this study

Primer sequence (lowercase sequences are restriction sites)	Plasmid	Protein
5′-GCCgagctcTTAACGCGCTTGACTGAGTAGGGTACT-3′ 5′-CCAggatccAGAACTTAAAGAGATATTAACTACCAG-3′	pETDuet IflAGAF1	IflA GAF1
5′-GCCgagctcTTAGCGACTGCTTTCATATAATTGTGC-3′ 5′-CCAggatccAGACTACAAACTTACCTTGCAAACTAT-3′	pETDuet IflAGAF3	IflA GAF3
5′-GCCgagctcTTAGCGACTGCTTTCATATAATTGTGC-3′ 5′-CCAggatccAATGACAGCAGTGACTGAGTACTCACA-3′	pETDuet IflA608	IflA608
5′-GCCgagctcTTAGCGACTGCTTTCATATAATTGTGC-3′ 5′-TATTATCGAGCAGTAGACTCTGCCCATATTCAATATTTAACAGCA-3′ 5′-TGCTGTTAAATATTGAATATGGGCAGAGTCTACTGCTCGATAATA-3′ 5′-CCAggatccAATGACAGCAGTGACTGAGTACTCACA-3′	pETDuet IflA608C141A	IflA608 C141A
5′-GCCgagctcTTAGCGACTGCTTTCATATAATTGTGC-3′ 5′-CCATTAGAAGCCTTCCCTGAAGACTATGCTCGTCTTTAC-3′ 5′-TTCAGGGAAGGCTTCTAATGGGGTTTTCATCCCCAA-3′	pETDuet IflA608C511A	IflA608 C511A
5′-CCAggatccAATGACAGCAGTGACTGAGTACTCACA-3′ 5′-GCCgagctcTTAGCGACTGCTTTCATATAATTGTGC-3′ 5′-AACAATGTAGCTTCCGAAGATTTGAATCCTGCTCATCGAGAATTT-3′ 5′-AAATTCTCGATGAGCAGGATTCAAATCTTCGGAAGCTACATTGTT-3′ 5′-CCAggatccAATGACAGCAGTGACTGAGTACTCACA-3′	pETDuet IflA608C539A	IflA608 C539A
5′-CCATTAGAAGCCTTCCCTGAAGACTATGCTCGTCTTTAC-3′ 5′-TTCAGGGAAGGCTTCTAATGGGGTTTTCATCCCCAA-3′ 5′-GCCgagctcTTAGCGACTGCTTTCATATAATTGTGC-3′ 5′-TATTATCGAGCAGTAGACTCTGCCCATATTCAATATTTAACAGCA-3′ 5′-TGCTGTTAAATATTGAATATGGGCAGAGTCTACTGCTCGATAATA-3′ 5′-CCAggatccAATGACAGCAGTGACTGAGTACTCACA-3′	pETDuet IflA608C141AC511A	IflA608 C141A C511A
5′-AACAATGTAGCTTCCGAAGATTTGAATCCTGCTCATCGAGAATTT-3′ 5′-AAATTCTCGATGAGCAGGATTCAAATCTTCGGAAGCTACATTGTT-3′ 5′-GCCgagctcTTAGCGACTGCTTTCATATAATTGTGC-3′ 5′-TATTATCGAGCAGTAGACTCTGCCCATATTCAATATTTAACAGCA-3′ 5′-TGCTGTTAAATATTGAATATGGGCAGAGTCTACTGCTCGATAATA-3′ 5′-CCAggatccAATGACAGCAGTGACTGAGTACTCACA-3′	pETDuet IflA608C141AC539A	IflA608 C141A C539A
5′-AACAATGTAGCTTCCGAAGATTTGAATCCTGCTCATCGAGAATTT-3′ 5′-AAATTCTCGATGAGCAGGATTCAAATCTTCGGAAGCTACATTGTT-3′ 5′-GCCgagctcTTAGCGACTGCTTTCATATAATTGTGC-3′ 5′-CCATTAGAAGCCTTCCCTGAAGACTATGCTCGTCTTTAC-3′ 5′-TTCAGGGAAGGCTTCTAATGGGGTTTTCATCCCCAA-3′ 5′-CCAggatccAATGACAGCAGTGACTGAGTACTCACA-3′	pETDuet IflA608C511AC539A	IflA608 C511A C539A
5′-GCCgagctcTTAGCGACTGCTTTCATATAATTGTGC-3′ 5′-AACAATGTAGCTTCCGAAGATTTGAATCCTGCTCATCGAGAATTT-3′ 5′-AAATTCTCGATGAGCAGGATTCAAATCTTCGGAAGCTACATTGTT-3′ 5′-CCATTAGAAGCCTTCCCTGAAGACTATGCTCGTCTTTAC-3′ 5′-TTCAGGGAAGGCTTCTAATGGGGTTTTCATCCCCAA-3′ 5′-TATTATCGAGCAGTAGACTCTGCCCATATTCAATATTTAACAGCA-3′ 5′-TGCTGTTAAATATTGAATATGGGCAGAGTCTACTGCTCGATAATA-3′ 5′-CCAggatccAATGACAGCAGTGACTGAGTACTCACA-3′	pETDuet IflA608C141AC511AC539A	IflA608 C141A C511A C539A