Electronic Supplementary Information:

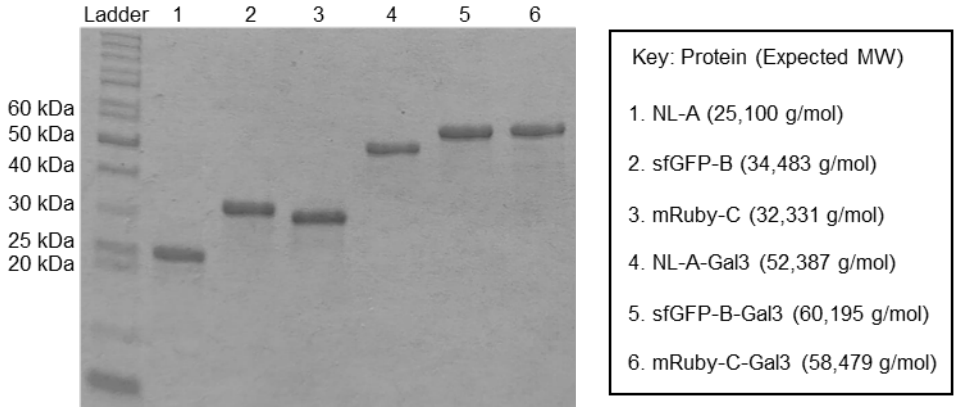


Figure S1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie staining of fusion proteins alongside a protein ladder.

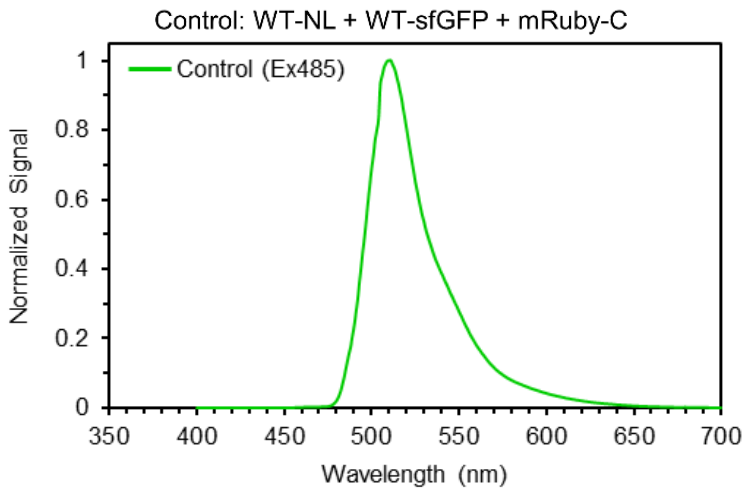


Figure S2. Normalized emission spectra of the control protein mixture upon excitation with 485 nm wavelength.

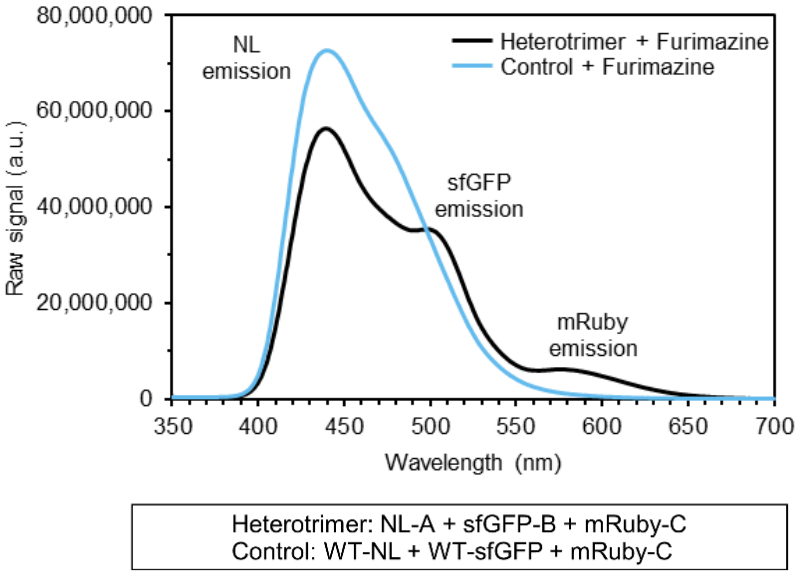


Figure S3. Raw emission spectra of the heterotrimer and control protein mixture upon addition of furimazine (black trace and blue trace, respectively).

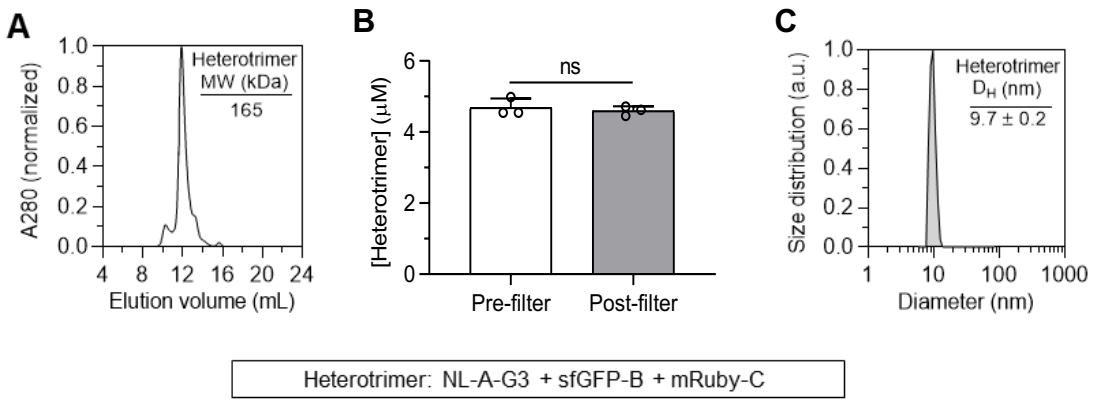


Figure S4. Approximation of Heterotrimer (NL-A-Gal3 + sfGFP-B + mRuby-C) native size. (A) Approximate native molecular weight measured by size-exclusion chromatography. (B) Molar concentration of Heterotrimer before and after passing through a 0.2-micron syringe filter for (C) dynamic light scattering experiments that measure hydrodynamic diameter (D_H). In C, mean +/- S.D. technical triplicate.

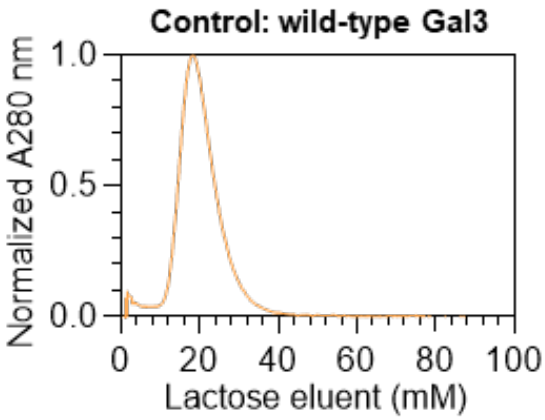


Figure S5. Lactose affinity chromatography trace of wild-type Gal3. The maximum signal of this trace is indicated in Figures 4-6 (vertical red dashed lines) showing wild-type Gal3 as a control to compare the lactose affinity binding properties of the fusion protein constructs.

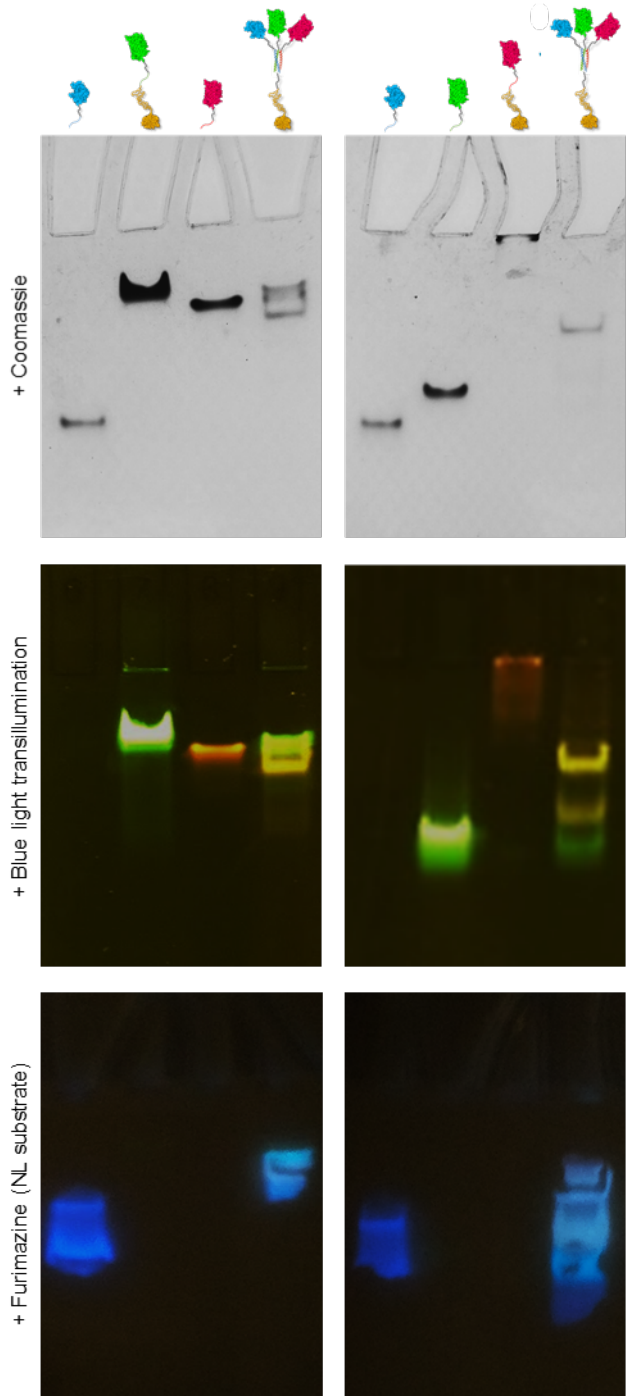


Figure S6. Native PAGE of NL-A mixed with sfGFP-B-Gal3 and mRuby-C (gels in the left column) or with sfGFP-B and mRuby-C-Gal3 (gels in the right column). Each combination of fusion proteins can result in co-localization of NL luminescence with sfGFP and mRuby fluorescence as well as an assembly with 1 Gal3 domain.

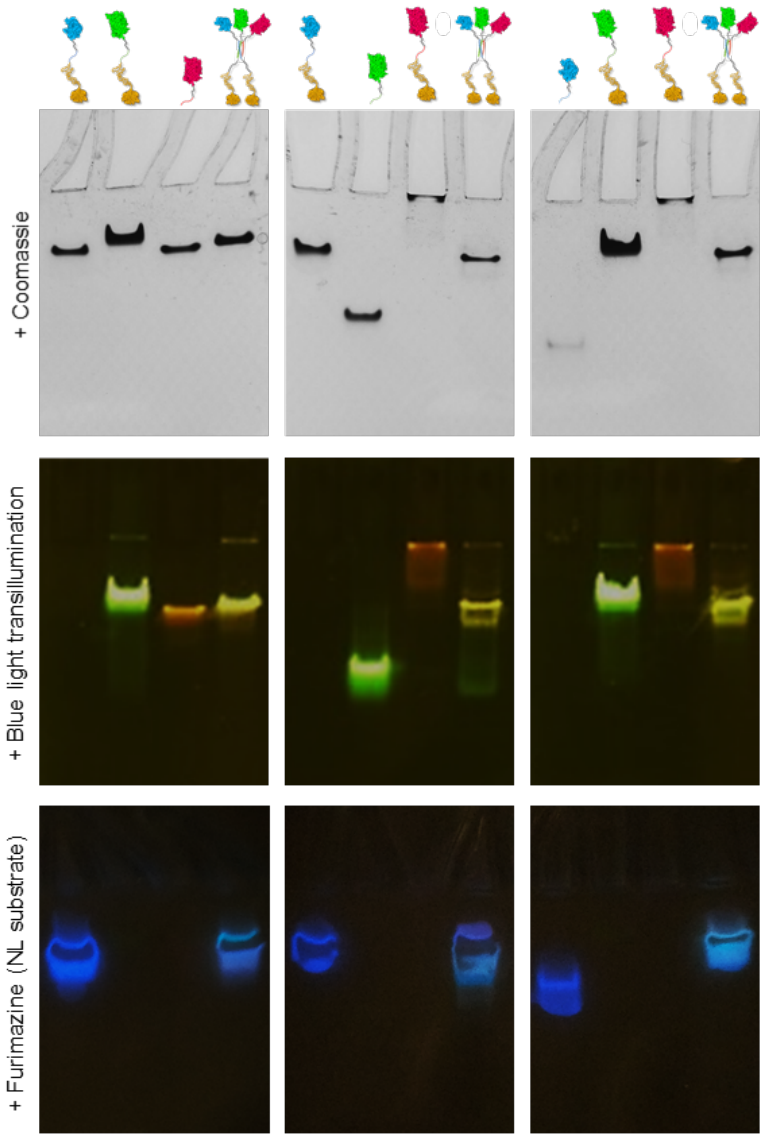


Figure S7. Native PAGE of NL-A-Gal3 mixed with sfGFP-B-Gal3 and mRuby-C (gels in the first column) or with sfGFP-B and mRuby-C-Gal3 (gels in the second column), while NL-A without Gal3 is mixed with sfGFP-B-Gal3 and mRuby-C-Gal3 (gels in third column). Each combination of fusion proteins can result in co-localization of NL luminescence with sfGFP and mRuby fluorescence as well as an assembly with 2 Gal3 domains.

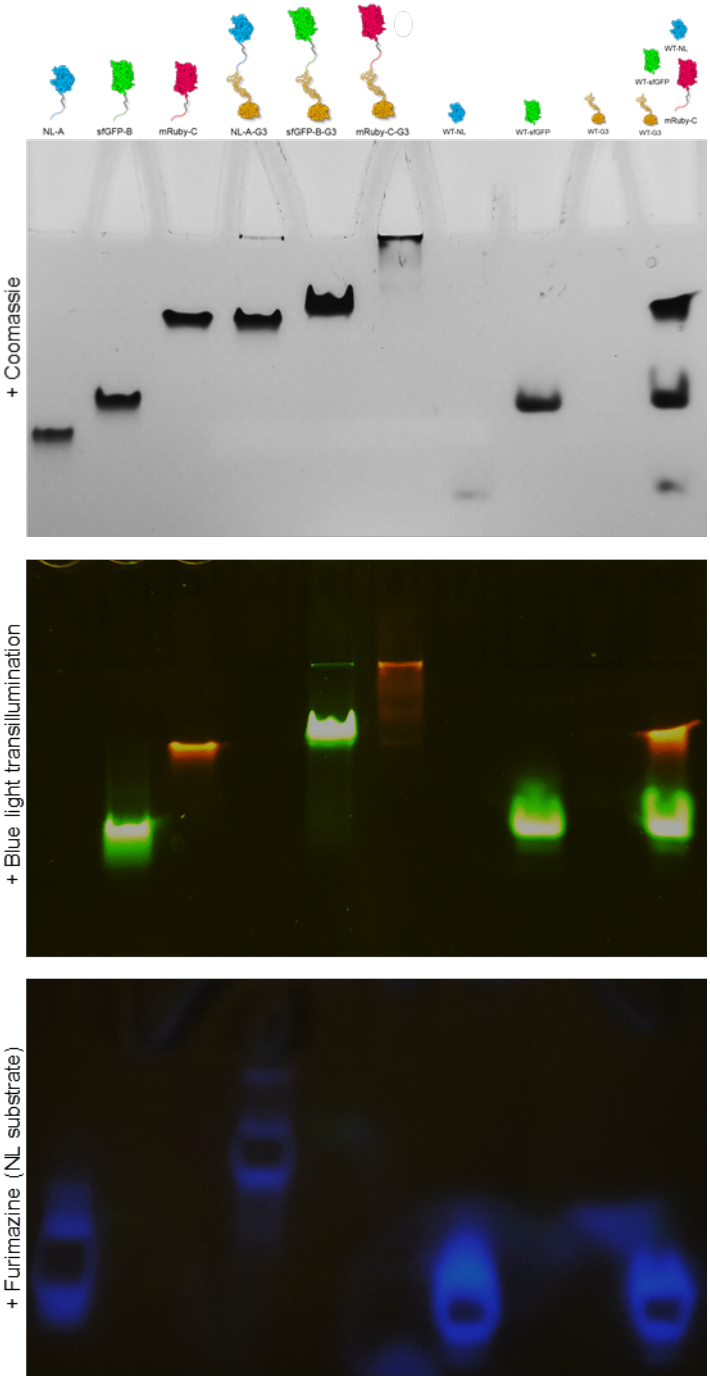


Figure S8. Control native PAGE experiment with wild-type variants of fusion proteins, which were performed to assess non-specific protein co-assembly.

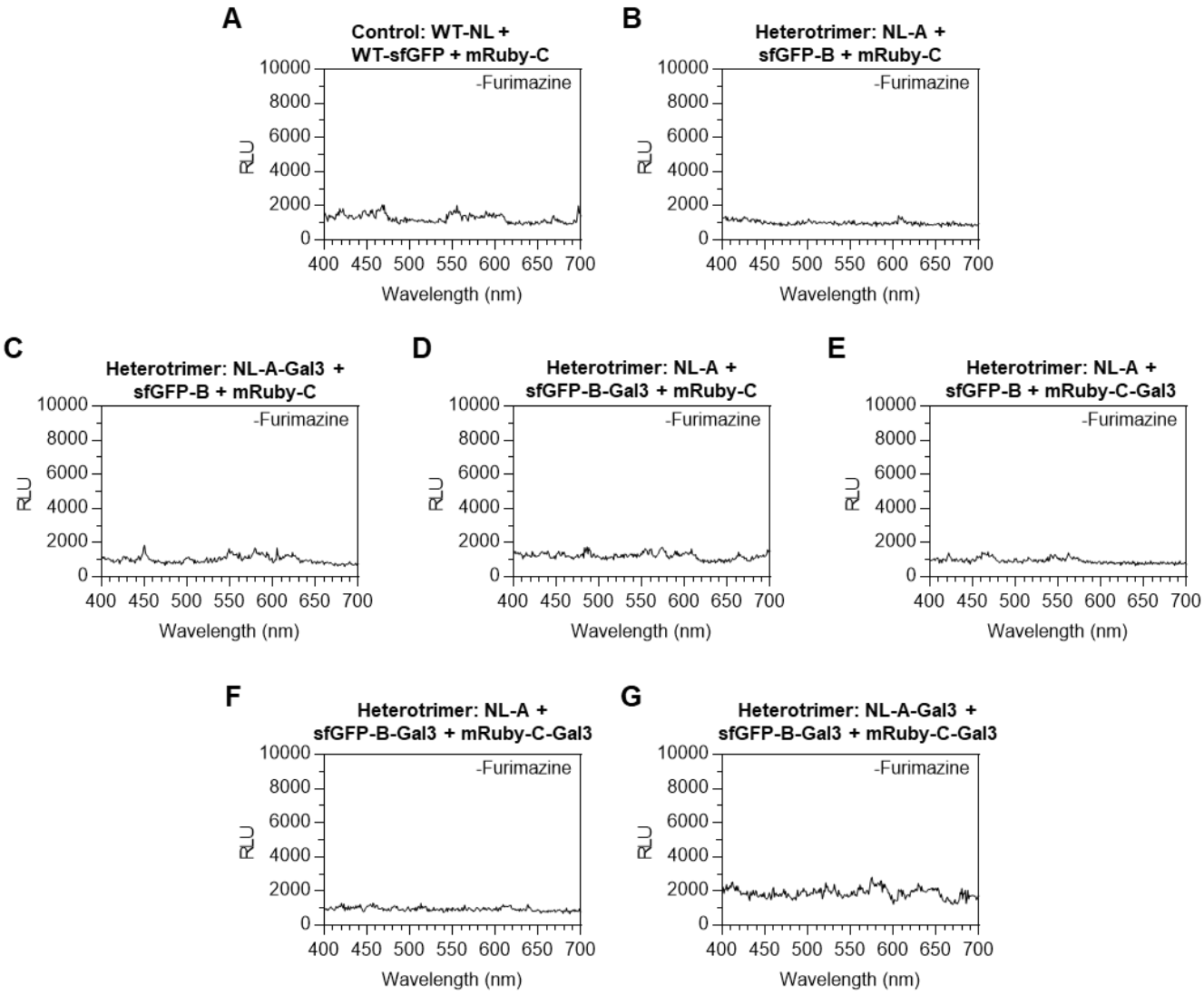
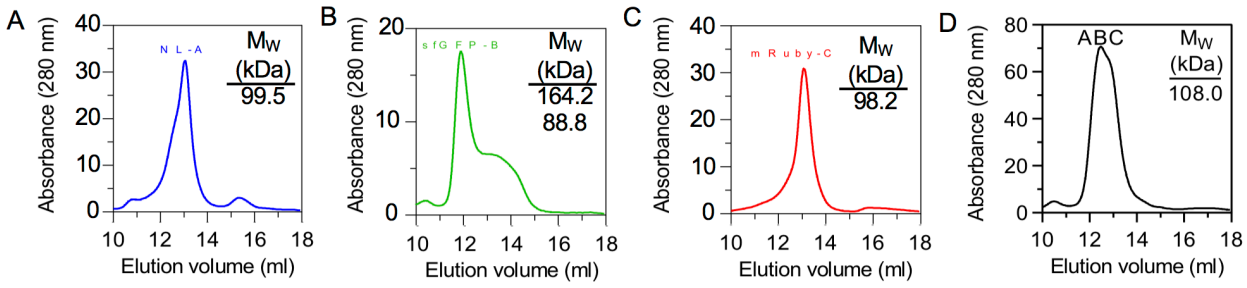


Figure S9. Background signal to BRET/FRET experiments in (A-B) Figure 3, (C) Figure 4, (D-E) Figure 5, and (F-G) Figure 6.



Empirical:

Sample	V_e	K_{av}	log(MW)	MW (Da)
Trial NL-A	13.05	0.258	4.998	99,483
Trial sfGFP-B	11.9	0.177	5.215	164,174
	13.31	0.277	4.949	88,830
Trial mRuby-C	13.083	0.261	4.992	98,063
Trial ABC	12.86	0.245	5.034	108,067

Theoretical:

Sample	V_e	K_{av}	log(MW)	Theor. MW (kDa)
Expected NL-A	16.21	0.481	4.400	25.1
Expected sfGFP-B	15.48	0.430	4.538	34.5
Expected mRuby-C	15.62	0.440	4.512	32.5
Expected ABC	13.22	0.270	4.966	92.1

Figure S10. Size-exclusion chromatography traces of (A) NL-A, (B) sfGFP-B, (C) mRuby-C, and (D) an equimolar ternary mixture of all three proteins. Bottom: tables of the measured elution volume, partition coefficient, log(MW) determined from SEC standards, and empirical MW calculated from log(MW), or theoretical MW calculated from amino acid sequence. Partition coefficient Kav = (Ve - Vo)/(Vt - Vo) where Ve is elution volume, Vo is column void volume, Vt is total column volume.

Genetic sequences

NL-A:

CCATGGCGGTCTTCACACTCGAAGATTTCGTTGGGGACTGGCGACAGACAGCCGGCTACAACCTG
GACCAAGTCCTTGAACAGGGAGGTGTGTCCAGTTTGTTTCAGAATCTCGGGGTGTCCGTAACTCC
GATCCAAAGGATTGTCCTGAGCGGTGAAAATGGGCTGAAGATCGACATCCATGTCATCATCCCGT
ATGAAGGTCTGAGCGGCGACCAAATGGGCCAGATCGAAAAAATTTTTAAGGTGGTGTACCCTGTG
GATGATCATCACTTTAAGGTGATCCTGCACTATGGCACACTGGTAATCGACGGGGTTACGCCGAA
CATGATCGACTATTTCGGACGGCCGTATGAAGGCATCGCCGTGTTCGACGGCAAAAAGATCACTG
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GATCCGGCGGCGGCAGCGGCGGCAGCGGCGGCGGCGGCAGCGGCGGCAGCGGCGCGGAGAT
CGCGGCGATTGAGTACGAACAGGCGGCGATCAAAGAGGAGATCGCGGCGATTAAGGACAAAATC
GCGGCGATTAAAGAATATATCGCGGCGATTGAATTCCACCACCACCACCACCACTGACTCGAG

sfGFP-B:

CCATGGCGTCCAAAGGAGAAGAGCTGTTCACTGGAGTGGTACCAATACTTGTGGAGTTGGACGGA
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mRuby-C:

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NL-A-G3:

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sfGFP-B-G3:

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mRuby-C-G3:

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Amino acid sequences

NL-A:
MAVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSG
DQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITVTGTLWNGNKII
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sfGFP-B:
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mRuby-C:
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EESDNEMFVVQREHAVAKFAGLGGGGSGGGSGGSGGGGSGGSGAEIAAIKYKQAAIKNEIAAIKQEIA
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NL-A-G3:
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sfGFP-B-G3:
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mRuby-C-G3:
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TFIKYPKGIPDFFKQSFPEGFTWERVTRYEDGGVITVMQDTSLEDGCLVYHAQVRGVNFPSNGAVMQK
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