Electronic Supplementary Material (ESI) for Molecular BioSystems

Split-superpositive GFP reassembly is a fast, efficient, and robust method for detecting protein - protein interactions *in vivo*

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Materials and Methods

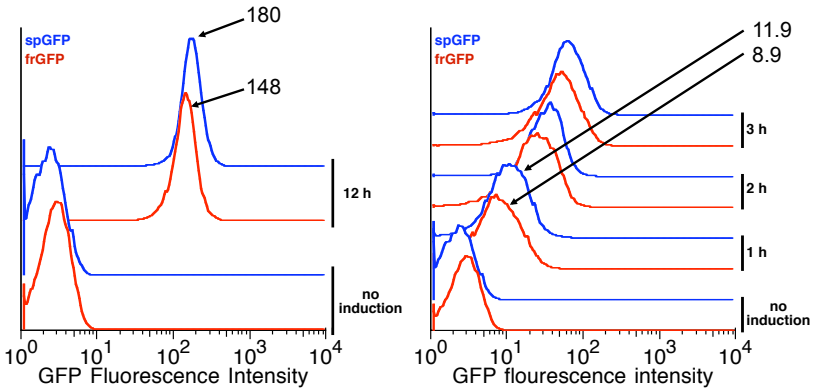
LB Miller's Broth – BD Biosciences
TOP10 one shot chemically competent E.coli – Life Tech
BL21 star E.coli – Life Tech
Bacteriological agar - Sigma Aldrich
Carbenicillin Disodium Salt – Growcells.com
L(+) arabinose – Calbiochem
Kanamycin – Fisher BioReagents
IPTG, dioxane free – Fermentas
MJ mini gradient thermal cycler – BioRad
Incubating / cooling shaker – VWR
Molecular imager gel doc XR+ system – BioRad
Flow Cytometer and High Speed Cell Sorter using a solid-state iCyt 488nm laser - MoFlo (Dako Colorado, Inc.)
Vent DNA Polymerase – New England Biolabs
10% Mini-Protean TGX precast gels - BioRad
Sonification - W-350 cell disruptor
J2-21 centrifuges – Beckman

Data Analysis and Additional Information

Analysis of flow cytometry data comparing split-frGFP and split-spGFP

All flow cytometry data was analyzed using FloJo software.

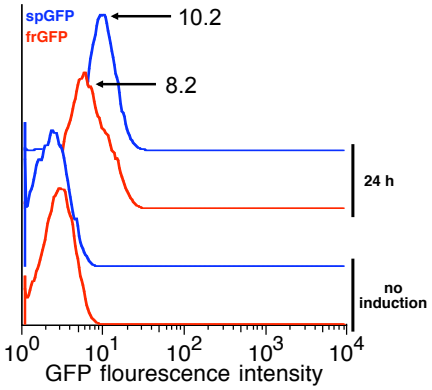
Figure S1. Mean cell fluorescence values are provided for Figure 4 in the manuscript. Cells were incubated at 25 °C for the indicated time



180 – 148 = 32
32/148 = 0.22
22% increase in spGFP cell fluorescence

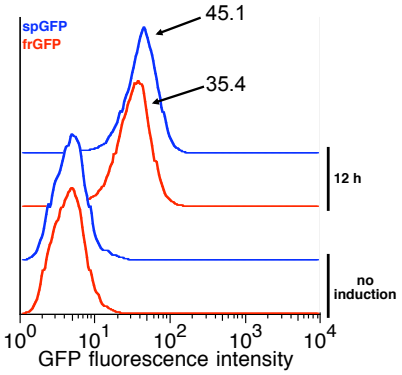
11.9 – 8.9 = 2
2/8.9 = 0.34
34% increase in spGFP cell fluorescence

Figure S2. Mean cell fluorescence values are provided for Figure 5 in the manuscript. Cells were incubated at 37 °C for the indicated time



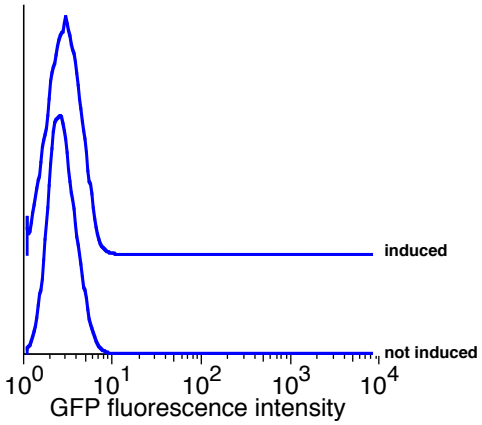
10.2 – 8.2 – 2
2/8.2 = 0.24
24% increase in spGFP cell fluorescence

Figure S3. Mean cell fluorescence values are provided for Figure 7 in the manuscript. Cells were incubated at 37 °C for the indicated time



45.1 – 35.4 = 9.7
9.7/35.4 = 0.27
27% increase in spGFP cell fluorescence

Figure S4. Flow cytometry data of *E. coli* expressing N-spGFP-Prb and C-sp-GFP-Gankyrin 24 hours after induction. Gankyrin is an ankyrin repeat protein with no known affinity for Prb. Thus, evolution of fluorescence in *E. coli* coexpressing N-spGFP-Prb and C-sp-GFP-Gankyrin is not an expected result. As anticipated, no cell fluorescence is observed, which shows that split-spGFP fragments to not reassemble in the absence of fused interacting pairs.



Amino acid sequence and net theoretical charge of split-GFP fragments

Superpositive (+34) GFP

N-spGFP

MGHHHHHHGGASKGERLFRGKVPILVELKGDVNGHKFSVRGEGKGDATRGKLTLKFICTTGKL
PVPWPTLVTTLTYGVQCFSRYPKHMKRHDFFKSAMPKGYVQERTISFKKDGKYKTRAEVKFE
GRTLVNRIKLKGRDFKEKGNILGHKLRYNFNSHKVYITADKR

positive amino acids = 13(Arg) + 24 (Lys) = 37
negative amino acids = 6(Asp) + 7(Glu) = 13
+24 total charge on N-scGFP

C-spGFP

KNGIKAKFKIRHNVKDGSVQLADHYQQNTPIGRGPVLLPRNHYLSTRSKLSKDPKEKRDHMVLL
EFVTAAGIKHGRDERYK

positive = 7 + 11 = 18
negative = 5 + 3 = 8
+10 total charge on C-spGFP

sg100GFP

N-sg100GFP

MASHHHHHHGASKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKL
PVPWPTLVTTLCYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFE
GDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVPIMADKQ

positive = 5 + 14 = 19
negative = 11 + 12 = 23
-4 total charge on N-sg100 GFP

C-sg100GFP

KNGIKVNFKTRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVL
LEFVTAAGITHGMDELYN

positive = 5 + 2 = 7
negative = 7 + 4 = 11
-4 total charge on C-sg100 GFP

fr-GFP

N-frGFP

MASHHHHHHGVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKL
PVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTISFKDDGNYKTRAEVKFE
GDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYITADKQ

positive = 4 + 14 = 18
negative = 11 + 12 = 23

-5 total charge on N-sg100 GFP

C-frGFP
KNGIKANFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLL
EFVTAAGITHGMDELYK

positive = 6 + 2 = 8
negative = 7 + 4 = 11
-3 total charge on C-sg100 GFP