1/9
(Janelle)

Cloning of individual CRY2/CIB1-CFP/YFP fusion proteins

Miniprepped pSB1C3-FRET2, pUC-CRY2-eCFP, pUC-CIB1-eYFP, pUC-CRY2-eYFP, pUC-CIB1-eYFP cultures.

Digested pSB1C3-FRET2 with XbaI/SpeI (Buffer B, 2 hours) and the pUC constructs with NcoI/HindIII (Buffer B, ON).

Ran gel to check pSB1C3-FRET2 digest - fragments at ~3kb and ~1.5kb not as expected.

2/9
(Janelle)

Cloning of individual CRY2/CIB1-CFP/YFP fusion proteins

Ran gel of all digests from yesterday. Purified CRY2/CIB1-eCFP/eYFP inserts but lost them on speedvac as left them on too long. Repeated digests from yesterday.

3/9
(Janelle)

Cloning of individual CRY2/CIB1-CFP/YFP fusion proteins

Ran gel of all digests from yesterday. Purified CRY2/CIB1-eCFP/eYFP inserts and ligated into NcoI/HindIII-cut pET28. Transformed into XLIB and plated on LB-kan.

4/9
(Janelle)

Cloning of individual CRY2/CIB1-CFP/YFP fusion proteins

Inoculated 2 colonies each from yesterday's transformations into 1.5mL LB-kan, incubated on 37degC shaker ON.

5/9
(Janelle)

Cloning of individual CRY2/CIB1-CFP/YFP fusion proteins

Miniprepped all cultures from yesterday and digested with NcoI/HindIII (Buffer B, ON).

6/9
(Henry)

Cloning of individual CRY2/CIB1-CFP/YFP fusion proteins

Ran gel of digests from yesterday - fragments not as expected (~6kb fragment in all digests, insert bands not correct length).

15/9
(Janelle)

Cloning of pET-eCFP-CRY2 and pET-CIB1-eYFP into submission plasmid for Registry
Digested pET-eCFP-CRY2 and pET-CIB1-eYFP with XbaI/SpeI (Buffer B, ON)

16/9
(Janelle)

Cloning of pET-eCFP-CRY2 and pET-CIB1-eYFP into submission plasmid for Registry
Digested pSB1C3 with XbaI/SpeI (Buffer B, 5 hours). Ran gel of all digests from yesterday and today. Cut out and purified pET-eCFP-CRY2 and pET-CIB1-eYFP inserts (~2.5 and 1.5kb respectively) and pSB1C3 vector backbone (~2kb). Ligated inserts into vector in duplicate, transformed into XLIB and plated on LB-chlor.

17/9
(Janelle)

Cloning of pET-eCFP-CRY2 and pET-CIB1-eYFP into submission plasmid for Registry
11 colonies observed for pET-eCFP-CRY2, >200 for pET-CIB1-eYFP and 5 for the pSB1C3 control. Inoculated 5 colonies of each into 1.5mL LB-chlor in 15mL falcon tubes, incubated ON on 37degC shaker.

18/9
(Henry, Janelle, Matt)

eCFP-CRY2 and CIB1-eYFP protein analysis

Purified eCFP-CRY2.

Concentrated 200uL of soluble fraction to 20uL (centrifuge 10 min 10000rpm). Added 5uL 4x SDS loading buffer to 15uL concentrated protein - insoluble and soluble eCFP-CRY2 and soluble CIB1-eYFP. Resuspended insoluble eCFP-CRY2 fraction in 40uL 4x SDS buffer and heated all solutions on 95degC hot plate for 5 minutes. Loaded 10uL to SDS-PAGE gel (lane 1: marker, 2: YFP (soluble), 3: CFP (soluble), 4: CFP (insoluble)). Ran gel for ~40 minutes at 150V. Placed in RO water, microwaved for 40 sec, replaced RO water and repeated 3 times.

Replaced RO water and left gel on shaker at RT for 10 minutes. Poured out water and submerged gel in Coomassie blue stain for 30 minutes. Destained with RO water for 1 hour before scanning gel.

Cloning of pET-eCFP-CRY2 and pET-CIB1-eYFP into submission plasmid for Registry
Miniprepped all cultures from yesterday and digested with XbaI/SpeI (Buffer B, 2 hours). Ran gel to check. pET-CIB1-eYFP colonies 1 and 5 showed expected fragments (~2kb and ~1.2kb); decided to submit pET-CIB1-eYFP colony 5. pET-eCFP-CRY2 digests did not show expected fragments.

Submission of pSB1C-PG6, pET-eCFP-CRY2 and pET-CIB1-eYFP to the Registry
Used NanoDrop spectrophotometer to measure plasmid concentrations. Diluted solutions to 25ng/uL (50uL) using nuclease-free water, dried down 10uL of diluted solutions of pSB1C-PG6 and pET-CIB1-eYFP in submission plate overnight in fume hood. (**Fig. 1**)

Plasmid	**Concentration (ng/uL)**	**Volume to add for 50uL 25ng/uL solution (uL)**
pSB1C-PG6	961.2	1.3
pET-CIB1-eYFP (1)	53.5	23.4
pET-CIB1-eYFP (2)	76.6	16.3
pET-CIB1-eYFP (3)	68.5	18.2
pET-CIB1-eYFP (4)	160.5	7.8
pET-CIB1-eYFP (5)	53.2	23.5
pET-eCFP-CRY2 (1)	60.7	20.6
pET-eCFP-CRY2 (2)	52.4	23.9
pET-eCFP-CRY2 (3)	51.6	24.4
pET-eCFP-CRY2 (4)	57.5	21.7