1/8
(Janelle)

No growth observed on ligation or control pET-FRET2 plates.

3/8
(Matt, Janelle)

Repeated pET-FRET2 ligation and transformation into XLIB.

4/8
(Spencer, Janelle)

Repeated NcoI/HindIII digest of pET16, pUC-PG4 and pUC-PG5 (from now on used HF-HindIII, Buffer E, 37degC ON).

SalI digest of pSB1C-PG6 and PSB3C-PG7 (4uL 10x Buffer D, 2uL SalI, 14uL water, 10uL plasmid, 37degC ON).

PCR amplification of Terminator: 40uL 2x Phusion, 4uL 10uM 5' Sal TrrnB, 4uL 3' Xho TrrnB, 1uL template pTre HisB, 3uL water. PCR cycle: 95degC 1 minute, 35x (95degC 30s, 55degC 30s, 72degC 30s), 72degC 5 min, store at 12degC.

5/8
(Spencer, Matt, Janelle)

SalI/XhoI digest of Terminator-PCR (37degC 1hr)

Gel electrophoresis and cut fragments from S/X PCR (300bp fragment), N/H pUC-PG4 (3kb fragment), N/H pUC-PG5 (3kb fragment), SalI PSB1C-PG6 (large fragment), SalI PSB3C-PG7 (large fragment) and N/H pET16 (large fragment).

Purified cut fragments.
Ligated N/H pUC-PG4 and N/H pUC-PG5 insert into N/H pET16, SalI/XhoI Terminator into SalI pSB1C-PG6 and pSB3C-PG7.

Transformed into XLIB and plated on LB-Amp (pET16) or LB-Chlor (pSB1C, pSB3C) for incubation 37deg ON.

6/8
(Spencer, Matt, Janelle)

Counted 40-50 colonies on pET-FRET1 and pET-FRET2 plates (no colonies on control).
Inoculated 2 colonies each in 5mL LB-Amp.

1 colony on pSB1C-PG6 plate (no colonies on control), inoculated in 5mL LB-Chlor. No colonies observed on pSB3C-PG7 plate or control.

Repeated ligation of SalI/XhoI Terminator into SalI pSB1C-PG6 (not enough Terminator to repeat pSB3C-PG7 ligation). Stored in freezer ON (subsequently discarded ligation reaction).

7/8
(Spencer, Janelle)

Repeated PCR amplification of Terminator. Cleaned up PCR product and digested with SalI/XhoI (15uL PCR Trrn, 3uL 10x Buffer E, 0.5uL SalI, 0.5uL XhoI, 37degC 2 hours).

Miniprepped pET-FRET1, pET-FRET2 and pSB1C-PG6 colonies. To check plasmids, NcoI/HindIII digest of pET-Fret1 and pET-FRET2 (Buffer E, 37degC 2.5 hours), XhoI/SalI digest of pSB1C-PG6 (Buffer D, 37degC 2.5 hours) as well as NcoI/BamHI digest of pSB1C-PG6 (Buffer E, 37degC 2 hours).

(during the period 8/8-20/8, Spencer did a lot of experimental work)

21/8
(Spencer, Janelle)

Cloning of pET-FRET2 into submission plasmid for Registry
Streaked 2 colonies of pET-FRET2 on LB-kan plate, incubated at 37degC ON.

22/8
(Janelle)

Cloning of pET-FRET2 into submission plasmid for Registry
Growth observed for both pET-FRET2 colonies, miniprepped pET-FRET2 plasmids. Digested pSB1C3 and pET-FRET2 with XbaI/SpeI (Buffer B, ON).

23/8
(Janelle)

Cloning of pET-FRET2 into submission plasmid for Registry
Ran digests on gel. pET-FRET(1) digest did not work, so repeated digest. Cut ~3kb fragment from XbaI/SpeI-cut pET-FRET2(2) and large fragment from pSB1C3, purified fragments. Ligated pET-FRET2(2) insert into pSB1C3, transformed into XLIB and plated on LB-chlor.

23/8
(Janelle)

Cloning of pET-FRET2 into submission plasmid for Registry

Inoculated 4 colonies of pSB1C3-FRET2(2) into 1.5mL LB-chlor. Incubated ON in 37degC shaker. Ran digest of pET-FRET1(1) on gel, did not work again.

24/8
(Janelle)

Cloning of pET-FRET2 into submission plasmid for Registry
Miniprepped pSB1C3-FRET2(2) and digested with Xba1/SpeI (Buffer B, 2 hours). Ran gel to check, fragments not expected size. Repeated XbaI/SpeI digest of pET-FRET2(2).

25/8
(Janelle)

Cloning of pET-FRET2 into submission plasmid for Registry
Ran gel of XbaI/SpeI digest, cut and purified ~3kb fragment. Ligated pET-FRET2(2) insert into pSB1C3, transformed into XLIB and plated on LB-chlor.

26/8
(Janelle, Henry)

Cloning of pET-FRET2 into submission plasmid for Registry
Inoculated 4 colonies of pSB1C3-FRET2(2) into 1.5mL LB-chlor. Incubated ON in 37degC shaker.

FRET assay

Cultured pET-FRET(1) and (2) in 10mL LB. Took 1mL aliquots and centrifuged (1min, 14600rpm) and resuspended pellets in water.

Conducted preliminary fluorescence spectrum (excitation 433nm with 5nm bandwidth, recorded emission spectrum from 470-540nm), (1) showed greater YFP peak.

Conducted second fluorescence spectrum to see YFP exclusively (excitation 480nm with 5nm bandwidth, recorded emission spectrum from 510-540nm).

Cultured (1) in 1L LB-kan. Incubated ON.

27/8
(Janelle, Henry)

Cloning of pET-FRET2 into submission plasmid for Registry
Miniprepped pSB1C3-FRET2(2) and digested with Xba1/SpeI (Buffer B, 2 hours). Ran gel to check, fragments not expected size.

FRET assay

Centrifuged 1mL of cells from large culture and resuspended in water.

Conducted measurement of fluorescence spectrum (excitation 433nm with 5nm bandwidth, recorded emission spectrum from 470-540nm), observed clear CFP peak. For second spectrum (excitation 480nm with 5nm bandwidth, recorded emission spectrum from 510-540nm) observed small YFP peak.

Induced 1L culture with 1mL 1M IPTG, incubated for 3.5 hours at 37degC. Incubated on shaker for 3 hours at 25degC.

Isolated 50mL of large culture then centrifuged all cultures. Stored pellet from 1L batch at -80degC. Cultured 50mL cells with empty pET vector for control.

28/8
(Henry, Janelle)

FRET assay

Lysed control cells and 50mL pellet from yesterday with bug buster detergent. Kept 5mL of crude lysate separate. Clarified lysate and kept supernatant.

Ran SDS-PAGE:
1. ladder
2. FRET crude
3. FRET soluble
4. control crude
5. control soluble

Left soluble FRET in the dark for 1 hour. Ran fluorescence measurement for soluble FRET (excitation 433nm with 5nm bandwidth, recorded emission spectrum from 470nm-540nm). Did not change upon blue-light induction (30 sec, max intensity).

Strong YFP peak observed, suggests association, but no CFP peak. Is CFP-CIB1 insoluble?

Left crude FRET in the dark for 40 minutes. Ran fluorescence measurement - comparable CFP and YFP peaks observed suggesting association. No difference observed after blue-light induction (1 minute max intensity).

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

Repeated PCR amplification of YFP. Cleaned up PCR product and digested with BglII/XhoI (Buffer D, ON).

Digested pUC-iGEM1 (CRY2-CreN) with BglII/SalI (Buffer D, ON) and pUC-IGEM2 (CIB1-CreC) with BamHI/XhoI (Buffer B, ON).

29/8
(Janelle)

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

Ran gels of all digests from yesterday.
Cut out and purified:

- ~2kb fragment from XbaI/SpeI-cut pSB1C3
- ~3kb fragments (one higher, one lower) from XbaI/SpeI-cut pET-FRET2(2)
- ~700bp fragment from XbaI/SpeI-cut eCFP
- ~3kb fragments from BglII/SalI-cut pUC-iGEM1 and BamHI/XhoI pUC-iGEM2

Ligated eCFP insert into both pUC-iGEM1 and pUC-iGEM2 vectors. Also ligated pET-FRET2(2) inserts into pSB1C3 vector. Transformed into XLIB and plated ON on LB-amp and LB-chlor respectively at 37degC .

Expected band absent from gel, thus repeated PCR amplification of YFP. Cleaned up PCR product and digested with BglII/XhoI (Buffer D, 2 hrs). Ran digest on agarose gel and cut out and purified bands at ~700kb.

30/8
(Janelle)

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

Ligated eYFP insert into both pUC-iGEM1 and pUC-iGEM2 vectors. Transformed into XLIB and plated ON at 37degC on LB-amp.

~100 colonies for one pSB1C3-FRET2(1) transformant, ~15 for the other (no colonies on control). Inoculated 4 colonies each into 1.5mL LB-chlor.

~10 colonies each for pUC-CRY2-eCFP and control, ~120 colonies for pUC-CIB1-eCFP and ~40 for control. Inoculated 4 colonies of each into 5mL LB-amp. (Mislabelled both pUC-CFP plates as CRY2 yesterday, numbered all cell cultures to keep track and check by digest.). Incubated ON in 37degC shaker.

31/8
(Janelle)

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

~10 colonies each for pUC-CRY2-eYFP and control, >100 for pUC-CIB1-eYFP and control.
Plated 3 colonies of each into 5mL LB-amp ON at 37degC.