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 Xbal/Spel (Buffer B, 2 hours) and the pUC constructs with Ncol/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 Ncol/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 Ncol/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 Xbal/Spel (Buffer B, ON)

16/9

(Janelle)

Cloning of pET-eCFP-CRY2 and pET-CIB1-eYFP into submission plasmid for Registry Digested pSB1C3 with Xbal/Spel (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 2 colonies of each into 1.5mL LB-chlor, incubated ON on 37degC shaker.

18/9

(Janelle)

Cloning of pET-eCFP-CRY2 and pET-CIB1-eYFP into submission plasmid for Registry Miniprepped all cultures from yesterday and digested with Xbal/Spel (Buffer B, 2 hours). Rangel to check.