1/7
(Spencer, Matt, Janelle)

Table 1. **Primers from Sigma-Aldrich.** RE recognition sequences underlined, YFP binding sites in bold.

3XhoI YFP	Tm = 71.2degC	ACTAGTCTCGAGT**TTACTTGTACAGCTCGTCCA**
5BglII YFP	Tm = 75.1degC	AGAGTAGATCTATG**GTGAGCAAGGGCGAGGA**

Table 2. YFP gene. Primer binding sites in bold.

GATTATTCGTCAGGCAGCAGATCAGGGCCTGAAAGCAAAACTGGTTAGCGGTGATGGTATTGTTA
GCAATGAACTGGCAAGCATTGCCGGTGATGCAGTTGAAGGCACCCTGAATACATTTGGTCCTGAT
CCGACCCTGCGTCCGGAAAATAAAGAACTGGTTGAAAAATTCAAAGCCGCAGGCTTTAATCCGGA
AGCATATACCCTGTATAGCTATGCAGCAATGCAGGCAATTGCGGGTGCAGCCAAAGCAGCAGGTA
GCGTTGAACCGGAAAAAGTTGCAGAAGCACTGAAAAAAGGTAGCTTTCCGACCGCACTGGGTGAA
ATCAGCTTTGATGAAAAAGGTGATCCTAAACTGCCTGGCTATGTGATGTATGAATGGAAAAAAGGA
CCGGATGGCAAATTCACCTATATTCAGCAGggtgga**GTGAGCAAGGGCGAGGA**GCTGTTCACCGGG
GTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCG
AGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCT
GCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGCCTGCAGTGCTTCGCCCGCTAC
CCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGC
GCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGA
CACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGG
CACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAAGAACGG
CATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCAC
TACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCT
ACCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGT
GACCGCCGCCGGGATCACTCTCGGCA**TGGACGAGCTGTACAAGTAA**GAATTCGAGCTCCCGGGT
ACCATGGCATGCATCGATAGATCCGGCTGCTAAC

NB: Purple is portion of a different gene, grey is YFP, uncoloured is linker/vector

2/7
(Spencer, Matt?)

???

3/7
(Spencer, Laura)

(p. 63) 1.2b, 1.3a Inoculation of pUC-PG3 and pUC-PG4 transformed XLIB into LB-Amp
2 colonies each of pUC-PG3 and pUC-PG4 transformed XLIB inoculated into 4mL of ampicillin-supplemented LB and incubated for 37degC overnight.

4/7
(Janelle)

(p. 63) 1.2b, 1.3a Purification of pUC-PG3 and pUC-PG4 plasmids
4uL of both pUC-PG3 and pUC-PG4 cultures miniprepped. 10uL of each pUC-PG4 miniprep digested with BamHI/XhoI (Buffer B, 2 hours) and run on a gel to check plasmids (did not have

enzymes for pUC-PG3 digest). Showed ~700bp band, should not appear as ligation of BglII-cut end of YFP and BamHI-cut end of pUC-PG2 destroys BamHI recognition sequence. Repeated pUC-PG4 digest with SalI/XhoI (Buffer D, two nights).

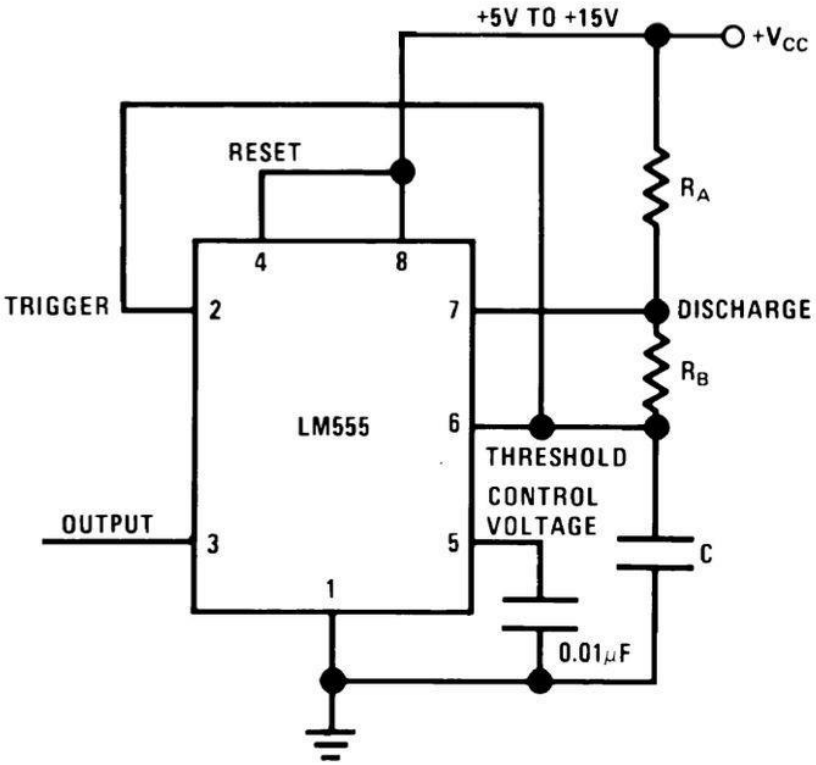
(p. 63) Purification of pET16-CRY2-CreN and pET16-CIB1-CreC controls
Each of 3 pET-CRY2-CreN and pET-CIB1-CreC cultures miniprepped (did not have enzymes to check by RE digest).

6/7
(Janelle)

(p.63)
Prepared 200mL 1% (w/v) agarose in TAE buffer. Ran gel of SalI/XhoI pUC-PG4 digest.

15/7
(Henry)

Design of blue light source timer circuit.
From the following reference:
http://www.nature.com/nmeth/journal/v7/n12/pdf/nmeth.1524.pdf
A blue light source at 450 nm and 4.5 mW with timed pulse capabilities was required for construction. I decided a 555 astable timer circuit was appropriate. I modelled the circuit from the following site:
http://www.instructables.com/id/555-Timer/step6/555-Timer-Astable-Mode-Circuit/
The circuit shown below was the fundamental design and incorporates a comparator / flip-flip combined in the IC where the capacitor charge time to a threshold voltage controls high and low times.



The theory gives the following relations for varied resistance (Ra, Rb) and Capacitance (C) where (∝) means proportional. This allows a completely variable timer system.

High time ∝ C*Ra*Rb

Low time ∝ C*Rb

16/7
(Henry)

Construction of blue light source timer circuit.
The above circuit was modified to include variable 1M Ohm logarithmic resistors (pots) at Ra and Rb. All components were purchased from JayCar. After frying some pots at low resistance due to current overload (sorry), a 10K resistor was included in series to protect all pots. A relay circuit was connected to the 555 output to control an exterior circuit that will connect to the blue light source running at 6V and high current. The 555 runs at 9V and low current. The entire apparatus was assembled on a breadboard and boxed with a double pole triple throw switch to allow constant on, off or timer options. A pot was included on the external circuit to control light intensity (see below).

The external circuit was constructed from four 3V LEDs at 470 nm which were wired with two in series and connected to a 6V source.

27/7
(Henry, Janelle, Matt)

Ran gel electrophoresis of 3 pET-CIB1-CreC samples and 3 pET-CRY2-CreN samples (from previous cultures of 3 colonies from respective plates) after cutting with NcoII and HindIII restriction enzymes in buffer B solution. As expected all pET-CRY-CreN samples gave a short fragment of 1.8kb but pETCIB1-CreC samples either didn't show RE action or gave a short fragment of 1.5kb instead of expected 1.2kb.

4 new colonies from pET-CIB1-CreC plate were selected to test again with gel electrophoresis. *They were put into amp liquid culture.

We also cut pUC-PG4 with NcoII and HindIII for ligation at a later day with pET16 and left overnight to incubate.

28/7
(Spencer, Henry, Janelle, Matt)

Ran gel of N/H pUC-PG4 from yesterday and B/X pUC-PG3 fragment. Cut out the 3kb fragment of pUC-PG4 (insert) and pUC-PG3 large fragment (plasmid + CRY2, eYFP, CIB1). Purified these fragments using standard protocol.

Ligation

pUC-PG5 was constructed by ligating B/X pUC-PG3 fragment from gel abve and B/SalI eCFP. We used a control of only plasmid without new insert (B/S eCFP).

pET-FRET1 was constructed by ligating cut N/H pET16 vector and N/H pUC-PG4 isolated from gel above. Two controls of pET16 vector only and N/H pUC-PG4 insert only were ligated.

The above ligated structures were transformed into competent E. Coli and plated out onto ampicillin plates in 37 degree incubator.

Incubated colonies of pET-CIB1-CreC in liquid culture on 27/7 were miniprepped to isolate plasmid DNA and plasmid DNA was ran through gel electro. Strange results - but isolated short fragment (as seen in Lab Book) to use in ligation tomorrow.

Spencer cut pET16 and pET28 vectors with N/H.

29/7
(Connor, Matt, Janelle, Henry)

Ran gel electrophoresis on cut pET16 and pET28 N/H vectors from yesterday. Cleaned long fragment from this gel as well as the isolated pET CIB1-CreC gel isolated yesterday.

4 colonies from pET16 pUC-PG4 N/H that were transformed and plated yesterday were used to inoculate 4 LB.AMP broths today. 2 colonies from the pUC-PG5 plate were also used to inoculate 2 LB.Amp liquid cultures. Cultures were placed in the incubator.

Cleaned cut pET16 fragments were ligated with CIB1-CreC fragments (also isolated today) and transformed in competent E. coli using heat shock.

30/7
(Janelle)

Miniprepped pET-FRET1 and pUC-PG5 using standard protocol.

Plate observations of pET16-CIB1-CreC : > 200 colonies
control 60 colonies
Inoculated 4x5mL Lb-amp solutions with pET16-CIB1-CreC and incubated in shaker incubater

DIgestion:
Digested pET-FRET and pUC-PG5 to check plasmids correctly ligated using NcoII and HindIII RE for both digestions and standard mastermix protocol (buffer B).
Digested at 37 degrees.

31/7
(Matt, Janelle)

Miniprep of each of the pET16-CIB1-CreC liquid cultures to isolate plasmid.
Digested plasmids from each pET16-CIB1-CreC cultures that were miniprepped. Colony 2 gave bizarre, multiple-stand gel reading - so we took this colony plasmid concentrate from miniprep today and cut with either NcoII/HindIII or NcoII/XhoII to determine if HindIII RE was at fault.

Isolated 3kb insert pUC-PG5 N/H fragment from the gel and cleaned using standard protocol.
Ligated 3kb insert from pUC-PG5 with pET16 N/H plasmid. Shared 2uL of buffer, vector, H20, ligase to a control of only insert, and a ligation of insert with pET16.

Ligation products of pUC+pET16 (forming pET-FRET2) were transformed into competent E. coli.