Tina Chen – Peking iGEM 2012 Experimental Log

June 27, 2012
*Transformation of LexA-VVD into Trans5-alpha competent cells.

July 04, 2012 – July 06, 2012
*Resuspension of part from iGEM parts registry - Plate 1; wells 7D and 2D
*Transformation into Trans5-alpha; plated and innoculated

July 10, 2012
*Picked single colonies; streaked on LB+Amp agar plates
*Mini-prep'd pYES2 (yeast plasmid) from Trans5-alpha competent cells (innoculated previously by QZ)

July 11, 2012
*rLuc (BBa_J52008) into Trans1T1 competent cells; plate on AK+ LB agar plates
*innoculated two (2) cultures of above transformation into 5mL of LB+Kan each

July 12, 2012
*Mini-prep'd cultures innoculated last night of rLuc in PSB1AK3

July 13, 2012
*Double digestion of rLuc with SpeI and EcoRI & pYES2 with EcoRI and XbaI
*Ran gel of above digests, using respective DNA ladders and agarose gel concentrations
*Gel purification of pYES2
*Crazy attempt at a DIY-Maxi-Prep of renilla luciferase and firefly luciferase with LJT

July 14, 2012
*Ligation of rLuc and pYES2
*Ran gel of previous day's gel purification to confirm existence
*Transformation of pYES2_rLuc into Trans5-alpha competent cells
*Innoculate more cultures of rLuc (pSB1AK3)

July 15, 2012
*Streaked out plates from previous day's transformation of pYES2_rLuc
*Innoculated two (2) cultures from pYES2_rLuc transformation from each plate (total four (4)) - 5mL LB+Amp each
*Mini-prep'd rLuc cultures from previous day
*Ran gel of rLuc mini-prep for confirmation
*Double digestion of rLuc in pSB1AK3 with SpeI and EcoRI
*Ran a gel of above rLuc digestion
*Double digestion of pYES2 with EcoRi and XbaI
*Gel purification of rLuc digestion

July 16, 2012
*Mini-prep'd rLuc_pYES2 (Trans5-alpha)
*Ran gel of rLuc double digest and pYES2 double digset again for confirmation
*Sent in for sequencing
*Double digestion of pYES2 with XbaI and EcoRI
*Innoculated ten (10) 5mL LB+Amp cultures of pYES2

*Ran gel of pYES2 digest and pYES2_rLuc digest for confirmation

July 17, 2012
*Mini-prep'd pYES2 cultures from previous day
*Double digest of pYES2 with EcoRI and XbaI
*Ran gel of pYES2 and pYES2_rLuc again for confirmation

July 18, 2012
*Double digest of pYES2 and rLuc again (with same enzymes as before)
*Ligation of pYES2 with rLuc
*Transformation into Trans5-alpha
*Plate on LB+Amp agar plates

July 21, 2012 - July 22, 2012
*Sequencing of rLuc in pYES2
*Mini-prep'd pYES2_rLuc cultures
*Ran gel of above mini-prep
*Double digested pYES2 with EcoRI and XbaI
*Ran Gel of above digestion

July 23, 2012
*SD-Ura and YEPD yeast media

July 26, 2012
*Transformation of pYES2_rLuc into BY4741 (S. Cerevisiae)

July 31, 2012
*Made more media for yeast cultures
*Recieved order of EnduRen and ViviRen from Promega (Luciferin-like substrate)
*Resuspension and dilution of the above according to given protocol
*Innoculate four (4) 5mL cultures of pYES2_rLuc (BY4741) in YEPD media

August 01, 2012
*Self-derived protocol for ViviRen and EnduRen due to lack of information on Promega protocol
*Diluted cultures of pYES2_rLuc to appropriate OD's and seperated samples into 96-well plates for assay
*Innoculate cultures for sequencing

August 03, 2012
*attempt the 96-well plate ViviRen/EnduRen assays again with different dilutions of cultures to different OD's
*Colony PCR of pYES2_rLuc
*Ran gel of above cPCR
*Transformed plasmid with R0051 promoter into Trans5-alpha; plate on LB+Amp; Innoculate

August 04, 2012
*Mini-prep'd R0051
*Double digest of R0051 with PstI and SpeI
*PCR of rLuc in pSB1AK3 using rLuc F&R
*Ran gel of rLuc PCR for confirmation
*Gel purification of rLuc PCR

*Double digest of rLuc PCR and R0051 with respective restriction enzymes
*Innoculate three (3) x LB+Amp of J23119 (promoter), three (3) x LB+Amp of J23100 (promoter)
*Innoculate four (4) x cultures of BY4741 (rLuc_pYES2) in YEPD (5 mL each), four (4) x cultures of same in SD-URA; incubate overnight in 30C shaker

August 05, 2012 - August 07, 2012
*Mini-prep'd J23100 (x3) and J23119 (x3)
*Ran gel of rLuc PCR Double Digest and R0051
*Gel purification of rLuc DD and J23100 DD, J23119 DD
*Ligation of rLuc with J23100 and rLuc with J23119
*Transformation into Trans5-alpha
*Innoculate at 37C shaker overnight (5mL LB+Amp)
*Miniprep'd R0051,J23100,J23119
*Transformation of J23100 and J23119 into Trans5-alpha
*cPCR of rLuc_pYES2 in BY4741; ran gel
* Plated above transformation and innoculated 5mL cultures

August 08, 2012
*Diluted BY4741 cultures into larger flasks, grown until night time to perform luminescence assay
*Luminescence assay with both ViviRen and EnduRen
*Innoculate new cultures from plates for future assays

August 09, 2012 - August 11, 2012
*Attempted a new method of luminescence assay in empty agar plates with same two substrates
*Repeated attempts; trouble shooting?

August 13, 2012
*Ran gel of ColEmCherry,SulAGFP,ColE408GFP(PSB1C3),ColE408(PSB3C5) (from LDY); then gel purification
*Point mutation using Takara MutanBEST Kit of the above constructs
*Ligation and transformation into Trans5-alpha competent cells

August 14, 2012 - August 16, 2012
*Plate > cPCR > liquid cultures of the above
*Ran gel of cPCR
*Mini-prep'd cultures from previous night
*Re-do of ColEmCherry (follow same outline as above); ligation, transformation

August 17, 2012
*Re-do of ColEmCherry outline again, failure to successfully transform. Modifications made.
*Streaked new plates for all of the previous transformations of ColE408GFP(1C3,3C5),PSulAGFP
*Send for sequencing

August 18, 2012 - August 20, 2012
*Strain lacking LexAVVD from Yang Yi, transformed ColE408GFP (IC3) and PSulAGFP seperately into self-made competent cells
*Transformation, plate, overnight in incubator 37C

August 22, 2012 - August 24, 2012
*Transformation of LexA408VVD plasmid and ColE408GFP plasmid into Yang Yi's strain

*Plates and cultures; liquid cultures in LB+CS media at 30C shaker incubator ~ 16-20 hours. LED exposure vs. covered in foil (no LED exposure)

August 24, 2012 - August 26, 2012
*Transformation of LexA408VVD plasmid and ColE408GFP plasmid into Trans5-alpha competent cells
*Plates and cultures; liquid cultures in LB+CS media at 30C shaker incubator ~ 16-20 hours. LED exposure vs. covered in foil (no LED exposure)

August 27, 2012 - August 29, 2012
*Failure to see proper luminescence in previous attempt, therefore repeat of the above.

August 30, 2012 - August 31, 2012
*Cleaned out some old stored plates and cultures
*Said my goodbyes!