Rough Timeline for QCB 301

Week of 10/14

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	Plasmids Obtain GAL + Far1-22 from Botstein Lab **Plasmids** Obtain construct from McClean Lab. **Prep** Plan out and order primers. **Prep** If needed, order oligos for weaker binding DBDs.	**Rap1** RNA purification and send to sequencing.	**Controls** If I have them already, transform yeast with GAL + Far1-22 plasmid. Also with only the blue light construct plasmids. **Shift Schedule** If this is done today, do the GAL experiments next Tuesday.	**Today or next week.** PCR amplify fragments for *Far1-22* and *Far-WT* from Botstein plasmid. **Today or next week.** PCR amplify fragments for *DBDs* from McClean plasmid.	**By today** Have obtained plasmids Planned recombination Ordered primers/oligos.	

Week of 10/21

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	PCR If not done yet, do PCR. Also amplify Far1 WT **mutPCR** If I want to do this, do it today (recombination later or tomorrow). **Recombination** Put together Far1-22/WT + DBD strains.	**GAL gradient** Transform yeast with GAL plasmid (Botstein). **Light Control** Transform yeast with two blue light plasmids. **GAL media** Set up dosages of galactose and glucose for gradient work.	**Construction** Store some cells from recombination. Extract plasmid and transform *E. coli*. **Positive Feedback** Plan primers to recombine zinc finger promoter with ACT1 promoter in plasmid.	**Mini-prep** Extract plasmid and purify Send to sequencing. **GALp gradient** Store cultures for next week. **Light Control** Store cultures for next week.	**Mini-prep** Do today if a day behind. **By today** Recombined plasmid strains. Grow up yeast; transfer plasmid to bacteria. Send to sequencing?	

Week of 10/28

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Tests this week If we have weak DBDs for Far1-22, also include in tests.	**GAL gradient** Taking transformed cells, set up several tubes of liquid culture to measure OD_{600} over time. Come in earlier today ($\sim 12-1:00$). **GAL gradient** Meanwhile add portioned gal/glu to cells and measure speed of cell arrest by microscopy. **Protein Stability** Measure when	**GAL** Yesterday's experiments should spill over to here. **Otherwise** Go over sequencing results and pick out cultures. **Tomorrow** Plan out the strains and the timing for tomorrow based on GAL results.	**Test Construct Arrest** Testing Far1-22, Far1WT, and (maybe) Far1-22 + weak DBDs. Grow at several light intensities and measure OD_{600} over time intervals. Plate in parallel and leave over several hours to assess rate of colony formation at different lights. Visualize cells from liquid culture by microscopy at time points given by GAL work.	**Test Construct Recovery** Using results from yesterday, choose light intensities that cause cell arrest, stimulate arrest, and then cover in foil for ~ 120 min to degrade Far1-22. Plate some cells and also visualize by microscopy.	**Construct Recovery** Check plates; visualize cells on slide to check for synchronization. Check for residual effects by measuring OD_{600} against a control and an uninduced sample. **By today** Have done at least GAL experiments and have construct arrest. Construct recovery would be optimal.	

Week of 11/04

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Light Control This week and next. **Alternatives** Start this week. Take any day to finish other work.	**RNA-seq** RNA-seq at 0, 60, 120 minutes of light intensity (IF a growth defect has been observed).	**Positive Feedback** Create fragment with zinc finger promoter (PCR) OR *weak DBD*. Linearize plasmid extracted from *E. coli* (sequencing step). **Prep** Blue light control cultures for tomorrow.	**Positive Feedback** Recombine to construct positive feedback CRY2 or CIB2 with DBD fragment and plasmid in yeast. Do so in the plasmid that does NOT contain Far1-22. **Blue Light** Taking control cells, measure growth relative to no light and no CRY2/CIB1 controls using OD_{600}.	**Blue Light** Microarrays for controls and intensities (Not sure of procedure. Consult.) Should also overlap into next week?	**Positive Feedback** Extract plasmid and transform *E.coli* (or transform next week). Also transform yeast with the other half of the plasmid, containing Far1-22 (so that I can do experiments on toxicity of the cryptochromes). **By today** Prepared to test alternates. Half done with the blue light controls. Have finished the construct tests.	

Week of 11/11

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Light Control Finish this week. **Alternatives** Positive feedback AND weak DBDs. Also this week. Take any day to finish other work.	**Light Microarrays** Do today if not last Thursday. **Positive Feedback** Extract plasmid from bacteria, miniprep, and send to sequence.	**Positive Feedback** Not waiting on sequencing, test growth by measuring OD_{600}, as with earlier constructs. Plate as well to see colony formation at different intensities.	**Alternatives and Recovery** Test recovery in similar way to construct in the alternative strategies. Plate cells and visualize by microscopy.	**Alternatives and Recovery** Check plates. Check cultures. Visualize cells to check for synchronization. Check residual effects as well by measuring growth.	**By today** Finished blue light testing. Tested alternate constructs.	

All weeks after this: analysis and buffer time.

If able to, continue experiments with western blots and/or further characterization of blue light effect on yeast with different promoters to test for toxicity (such as the ones in alternative experiments).