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 pCORE **Plasmids** Obtain construct from McClean Lab. **Prep** Plan out and order primers.	**GAL gradient** Set up plates and create dilutions. **Selection media** G418, 5-FOA, FAA, and SC -Leu -Trp plates.	**DO these when primers arrive:** **Today or next week.** PCR amplify fragments for *Far1-22* and *Far-WT* from Botstein plasmid. PCR amplify fragments from pCORE PCR amplify fragments for ZiF_{268} *DBD* from Noyes plasmid. **GAL + FAR1-22** Extract plasmid.	**By today** Have obtained plasmids Planned recombination Ordered primers/oligos. **FAR1-wt** Is the only PCR product we must have (to create the *GAL4DBD + FAR1WT* plasmid)	

Week of 10/21

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	Construction If not done yet, do the PCR. *(most likely today)* **Recombination** Put together Far1-WT + GAL4pr plasmid.	**Construction** Introduce CORE cassette into ZEV strain and plate on G418. **GAL gradient** Transform yeast with GAL plasmid (Botstein) and WT plasmid. **Light Control** Transform yeast with two blue light plasmids.	**PCR** If we have only done pCORE so far, do the rest here.	**Construction** Check G418 plates. Pick colonies. Plate onto FOA and FAA to test for cassette and location. **GAL FAR1-wt** Store culture, extract plasmid and transform. **GAL gradient** Store cultures for next week. **Light Control** Store cultures for next week.	**GAL FAR1-wt** Extract plasmid (MP) and send to sequencing	**By today:** FAR1-wt + GAL4pr plasmid sent to sequencing. Light control and GAL control transformed. pCORE replaced Trp1 in ZEV strain.

Week of 10/28
(Break)

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Tests this week GAL gradient tests: **Note:** Can spread this over several days, but must work with Denise's schedule, as it is break.	**Raffinose:** Grow up GAL cells overnight in raffinose. Should be FAR1-WT, FAR1-22, and non-transformed control. **Construction** Using the CORE containing strain, recombine CORE w/ new cassette. Plate on 5-FOA plates.	**GAL plates** Perform serial dilutions and plate into galactose and glucose plates. **Visually** Meanwhile add portioned gal/glu to cells and measure speed of cell arrest by microscopy. Once arrested, add glucose and visualize cells. **FACS** Induce all cells for 30 min (except one control group). Add propidium iodide. Set aside control group (one no glucose added and one never had galactose). Perform flow over at least 3-4 hours to measure cycle. (This might need to be on a separate day)	**Construction** From 5-FOA plates, pick colonies, plate on FAA(r) and G418(s).	**Transform** Several picked colonies with the blue light plasmids.	**Construction** Check plates. Both with plasmid and without.	**By today** Have done GAL experiments and have measured construct arrest in flow. Genomic construct has been made.

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.	**Test Construct Arrest** Testing Far1-22, Far1WT, and WT on β-estradiol containing plates. Plate in parallel and leave overnight 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 onto YPD (20% glucose) and also visualize by microscopy. **Set up** Materials to put together Blue light rig for next week.	**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. **Blue Light Rig** Work on blue light rig.	**Blue Light Rig** Work on blue light rig. Set up plates for blue light experiments.	**By today** Have finished the work with β-estradiol. Have finished the rig.	

Week of 11/11

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Light Control Do this week. **Light Experiment** Also this week, in parallel.	**RNA-seq** RNA-seq at 0, 60, 120, 240 minutes of light intensity (IF a growth defect has been observed). **Prep** Control blue light cultures and the rig for tomorrow.	**Prep** ALL blue light cultures and the rig for tomorrow. **Control BL** Taking control cells, measure growth relative to no light and no CRY2/CIB1 controls over time using OD_{600} and rig.	**Experiment BL** Taking both control cells, measure growth relative to no light and no CRY2/CIB1 controls over time using plates and rig. Set up some cells for 1-2 hours and then put back in dark.	**Control BL** Check plates and visualize Microarrays for controls and intensities Should also overlap into next week? **Exp. BL** Test for recovery	**By today** Half done with the blue light work.	

Week of 11/18

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Agenda Anything else that is needed.	**Light Microarrays** Do today if not last Thursday.	**If time, OD_{600}** Have raffinose starved cells from GAL group of experiments and perform a microtiter assay to measure growth overtime.			**By today** Finished blue light testing.	

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). If time, take GAL or other transformed cells, set up microtiter dishes to measure OD_{600} over time.

Parts List

Plates:

- 5-FOA (5-Fluoroorotic Acid)
- ClonNat + G418
- Synthetic Complete -LEU -TRP
- FAA (Fluoroanthranilic Acid)
- Glucose and Galactose gradient on minimal (glycerol)
- β-estradiol gradient on minimal (glycerol)

Plasmids:

- pGAL4p + FAR1-22 (Botstein)
- pCORE
- pMM159 (McClean, *CIB1 + GAL4AD*)
- pMM317 (McClean, *CRY2 + ZEV*)
- ZEV_{268}pr (Noyes, or QCB lab)

Chemicals:

- β-estradiol
- raffinose, glucose, galactose
- FAA, 5-FOA, G418 (Sigma Aldrich)
- propidium iodide (DNA staining) (Labs? Millipore)
- If time permits, α-factor.

General:

- QIAprep Miniprep Kit
- PCR mix (dNTPs, thermopol buffer, Taq DNA pol, EB)
- Replica plating materials
- Yeast transformation mix (LiAc, carrier DNA)
- Bacterial transformation materials

Strains:

- ZEV strain (Noyes)
- To be made, ZEV + CORE
- Bacterial competent cells
- If time permits, supersensitive (*Sst-*) yeast cells (for α-factor characterization).

Tools and access:

- FACS
- Sequencing
- Microscope
- 6x8 replica plating tool.
- Microtiter dish
- OD_{600} reading (microtiter)
- Microarray

Primers:

- 2x pCORE to around Trp1
- 2x *FAR1* and *FAR1-22* to ZEV and pCORE
- 2x *ZEVpr* to *FAR1/1-22* and pCORE
- If needed, 2x primers for N-degron tag.
- If needed, 2x primers for *cdc4*.

Blue Light Rig (From Open Wet Ware)

Physics storeroom:

- 0.1 μF capacitors
- 100 Ω 1/4 watt resistor
- 22 gauge solid core wire
- Toggle Switch
- LEDs (460nm emittance)

From Newark through Princeton Marketplace: Arduino: Newark part #$63W3545$*; lm7805: #*$09J6572$*; 12V power supply #*$40P7518$*; breadboard #*$99W1760$.