Overall Goals

- Creation of a plasmid containing *FAR1-22*/*FAR1-WT* driven by *GALpr*.
- Testing under a galactose and glucose gradient to determine level of activation required to stop cell cycle.
- Flow cytometry to measure DNA content after cell arrest.
- Creation of a genomic strain containing *FAR1-22*/*FAR1-WT* driven by $ZEV_{268}pr$.
- Testing under a β-estradiol gradient mimicking the gal/glu gradient.
- Determination of appropriate conditions for both β-estradiol and blue light induction.
- Blue light controls.

Steps for Plasmid Construction

- ☒ Miniprep and digest pMM86
- ☒ Miniprep pGAL-FAR1-22 (pTCN113)
- ☒ PCR amplify FAR1-22 fragment
- ☒ Colony PCR amplify FAR1-WT fragment
- ☐ Digest both fragments
- ☐ Gel extraction of fragments
- ☐ Ligation
- ☐ Transformation into bacteria
- ☐ Miniprep and sending to sequencing
- ☐ Transformation into yeast

Steps for Genomic Strain Construction

- ☒ Miniprep pMM299 (ZEVpr)
- ☒ Miniprep pCORE
- ☒ Miniprep pGAL-FAR1-22 (pTCN113)
- ☒ PCR amplify CORE fragment
- ☒ PCR amplify FAR1-22 fragment
- ☒ Colony PCR amplify FAR1-WT fragment
- ☒ Colony PCR amplify ZEVpr fragment
- ⊟ Transform ZEV yeast with CORE
- ☐ Transform ZEV+CORE with ZEVpr + FAR fragment
- ☐ Send to sequencing.
- ☐ Transform ZEV + FAR with the blue light plasmids

Other steps in the near future:

- ☐ Order sequencing primers.
- ☐ Build blue light rig.
- ☐ Transform ZEV strain w/ Blue light plasmids.
- ☐ Set up Galactose+Glucose stock.
- ☐ Plan for FACS experiment.