Expression Engineering Experiment

Day 1
- Lecture 1
  - eukaryotic gene expression
    - chromatin parts
    - euk exp'n + obstacles

Day 2
- Lecture 2
  - intro to yeast genetics
  - yeast SAGA complex
    - genetics then and now
    - SAGA genes

Day 3
- Lecture 3
  - IMRD (Susan Ruff)

Day 4
- Lecture 4
  - yeast genetic analysis

Day 5
- Lecture 5
  - measuring gene expression

Day 6
- Lecture 6
  - microarray analysis
    (Rebecca Fry)
Specific Aim 1
Identification of Melatonin-responsive yeast promoter

With your start up funds, you have

Part: BBa_J63001
Designed by Gonye Ay-Franks
enhanced version of EYFP, yeast-optimized YFP
Specific Aim 1
Identification of Melatonin-responsive yeast promoter
With your start up funds, you have

Specific Aim 2
Identification of light-insensitive yeast
With your start up funds, you have

RNA aptamer + Melatonin gives GFP signal

Specific Aim 2
Identification of light-insensitive yeast
With your start up funds, you have
deletion set 4000 strains, each with one nonessential gene replaced by KanMX

RNA aptamer + Melatonin gives GFP signal
Results for Specific Aim 2
Identification of light-insensitive yeast

What questions do you ask next?

Have identified two new genes
- MIT1 and MIT2: Mutation for Insomnia at Tech square
- mutants show light-insensitive melatonin-metabolism
- mit1 shows increased resistance to caffeine
- mit2 unable to grow at 37°C

- MIT1 and MIT2 are likely essential genes
- model: Mit1p and Mit2p indirectly regulate melatonin metabolism genes since no melatonin-responsive promoters were identified

RO1 Resubmission:
Preliminary Data Section

RO1 Resubmission:
New Specific Aims

1. Clone MIT1 and MIT2 and identify mutations that give rise to light insensitive phenotype
2. Examine changes in gene expression from mit1 and mit2 and double mutants when viable
3. Purify proteins associated with Mit1p and Mit2p