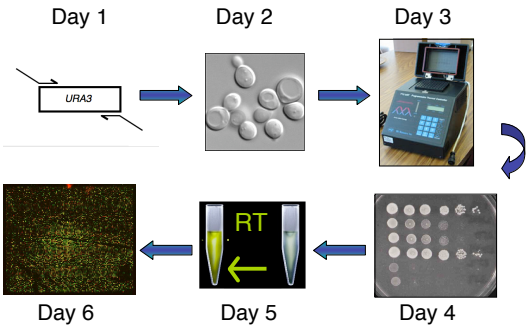
20.109: Expression Engineering

Spring 2007
Lecture 3
April 12th, 2007

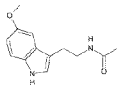
Expression Engineering Experiment

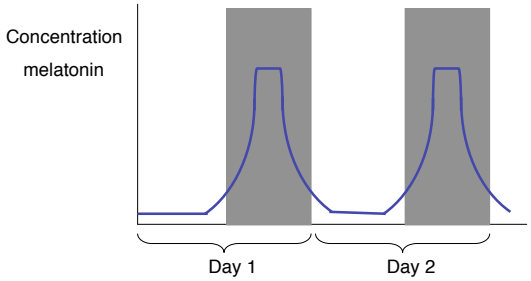


Expression Engineering Experiment

Lecture 1	Lecture 2
• eukaryotic gene expression ✓chromatin parts ✓euk exp'n + obstacles	• intro to yeast genetics • yeast SAGA complex ✓genetics then and now ✓SAGA genes
Lecture 3	Lecture 4
• IMRD (Susan Ruff)	• yeast genetic analysis
Lecture 5	Lecture 6
• measuring gene expression	• microarray analysis (Rebecca Fry)

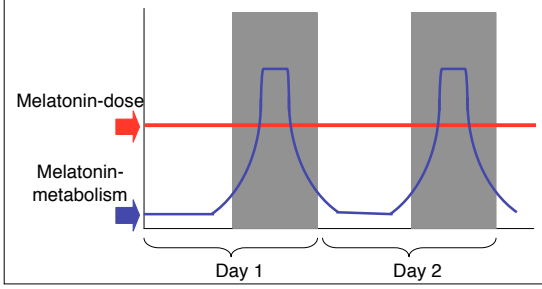
Human Sleep/Wake Cycle





Yeast Sleep/Wake Cycle

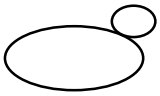
Postdoctoral publication: Melatonin-sensitive RNA aptamer



Specific Aim 1

Identification of Melatonin-responsive yeast promoter

With your start up funds, you have



Part:BBa_J63001

Designed by Caroline Ajo-Franklin

enhanced version of EYFP, yeast-optimized YFP

Specific Aim 1

Identification of Melatonin-responsive yeast promoter

With your start up funds, you have

Part:BBa_J63001

Designed by Caroline Ajo-Franklin

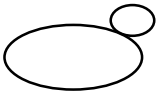
enhanced version of EYFP, yeast-optimized YFP

Specific Aim 2

Identification of light-insensitive yeast

With your start up funds, you have

RNA aptamer + Melatonin gives GFP signal



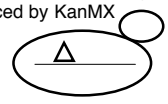
Specific Aim 2

Identification of light-insensitive yeast

With your start up funds, you have

RNA aptamer + Melatonin gives GFP signal

deletion set 4000 strains, each with one nonessential gene replaced by KanMX



Results for Specific Aim 2

Identification of light-insensitive yeast

What questions do you ask next?

RO1 Resubmission: Preliminary Data Section

Have identified two new genes

- *MIT1* and *MIT2*: **M**utation for **I**nsomnia at **T**ech square
- mutants show light-insensitive melatonin-metabolism
- *mit1* shows increased resistance to caffeine
- *mit2* unable to grow at 37°

- *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: 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