20.109 Expression Engineering

Spring 2007
Lecture 4
April 19th, 2007

Expression Engineering Experiment

Day 1  Day 2  Day 3

Day 6  Day 5  Day 4

Expression Engineering Experiment

<table>
<thead>
<tr>
<th>Lecture 1</th>
<th>Lecture 2</th>
</tr>
</thead>
</table>
| - 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  
  ✓ yeast circadian rhythms |
| Lecture 5 | Lecture 6 |
| - measuring gene expression | - microarray analysis  
  (Rebecca Fry) |
Yeast Sleep/Wake Cycle

Postdoctoral publication: Melatonin-sensitive RNA aptamer

RO1 Resubmission: New Specific Aims

1. Clone MIT1 and MIT2
2. Examine changes in gene expression from mit1 and mit2 mutants
3. Purify proteins associated with Mit1p and Mit2p

REVISED: Specific Aim 1
Clone MIT1 and MIT2

MIT1 = HOS1
deactylase

MIT2 =? S. c. LSD1
demethylase?

SET7/9
LSD1
HAT/MLL
LSD1/
HDAC

Clone MIT1 and MIT2

2. Examine changes in gene expression from mit1 and mit2 mutants

3. Purify proteins associated with Mit1p and Mit2p

- Contaminants
- Protease cleavage site
- Calmodulin binding peptide

www.orbm.cnrs.fr

RO1 Resubmission: New Specific Aims

- Clone MIT1 and MIT2

2. Examine changes in gene expression from mit1 and mit2 mutants

- Purify proteins associated with Mit1p and Mit2p

DNA

RNA

Protein

ChiP

Microarray

Northern/qPCR

SDS-PAGE

Activity Assay

Microarray

the array

http://www.youtube.com/watch?v=SBOw71nMNNU

http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html
Microarray sample preparation

- two samples to compare
- isolate RNA
- reverse transcribe and label

http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html

Microarray fluorescent tags

Cy3 ex em
Cy5 ex em

www.qubitsystems.com

Microarray wash and scan

http://www.bio.davidson.edu/people/macampbell/strategies/chipsintro.html
Microarray controls

Self-Self Microarrays
- S. cerevisiae

Dye Swap Microarrays
- S. cerevisiae

Microarray indirect labeling method

http://www.genisphere.com/about_3dna.html

Summary

MIT1, MIT2