Module 2: Expression Engineering

20.109
Lecture 1
October 11th, 2007

Eukaryotic Gene Expression

Obstacles to expression

Transcription

Eukaryotic Gene Expression

mRNA processing

Export
Translation
DNA---RNA---Protein

This movie was taken over a 12-hour period.

Movie credit: Kristina Yu, © Exploratorium, www.exploratorium.edu

Expression Engineering
Pressure Points

1. mRNA abundance
   - Ribosome density and occupancy
   - Protein concentration

2. transcription
   - processing
   - export
   - degradation

microRNA translation folding modification localization

“Quantification of protein half-lives in the budding yeast proteome”

How do you grow mammalian cells?

From Freshney’s “Culture of Mammalian Cells”
Culturing MES cells

Expression Engineering Experiment

RNA interference vocabulary

Transfection

Dicer

siRNA

RISC
### Expression Engineering Experiment

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<td>- intro to cell culture</td>
<td>- transfection</td>
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<td>- intro to gene expression/RNAi</td>
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<td>- off-target/nonspecific RNAi</td>
<td>- Writing lecture</td>
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<td>(Neal Lerner)</td>
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<td>- measuring gene expression</td>
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<td>Lecture 7</td>
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<td>- high throughput technologies or RNAI applications (no lab)</td>
<td>- review of your data</td>
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### Summary

1. Eukaryotic gene expression
2. Cell culture basics
   - 2a. cells in the lab
   - 2b. cells reaction times require Δ protein
3. RNAi features
   - 21bp siRNAs
   - Dicer
   - RISC
   - bind and destroy target mRNA