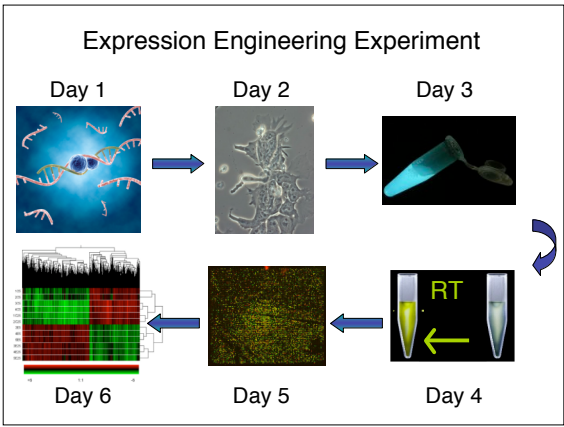


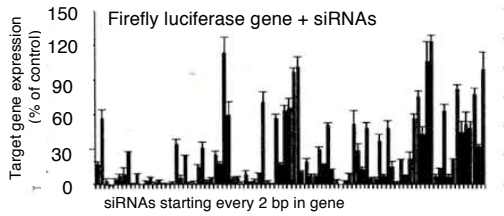
Module 2: Expression Engineering

 20.109
Lecture 3
 October 18th, 2007



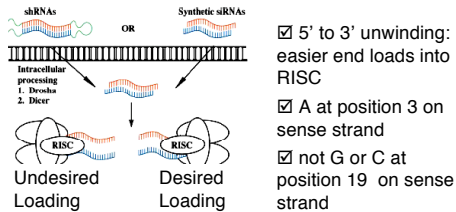
Expression Engineering Experiment	
<p style="text-align: center;"><u>Lecture 1</u></p> <ul style="list-style-type: none"> • intro to cell culture • intro to gene exp'n/RNAi 	<p style="text-align: center;"><u>Lecture 2</u></p> <ul style="list-style-type: none"> • transfection • luciferase
<p style="text-align: center;"><u>Lecture 3</u></p> <ul style="list-style-type: none"> • off-target/nonspecific RNAi 	<p style="text-align: center;"><u>Lecture 4</u></p> <ul style="list-style-type: none"> • Writing lecture (Neal Lerner)
<p style="text-align: center;"><u>Lecture 5</u></p> <ul style="list-style-type: none"> • measuring gene express'n 	<p style="text-align: center;"><u>Lecture 6</u></p> <ul style="list-style-type: none"> • microarray analysis (Rebecca Fry)
<p style="text-align: center;"><u>Lecture 7</u></p> <ul style="list-style-type: none"> • high throughput technologies or RNAi applications (no lab) 	<p style="text-align: center;"><u>Lecture 8</u></p> <ul style="list-style-type: none"> • review of your data

siRNA in practice: Design ambiguities



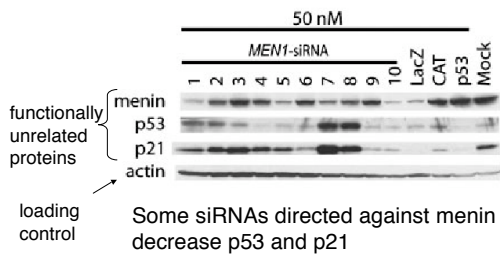
Reynolds et al Nature Biotech 2004

siRNA: design rules



- Empirically determined
- Mechanism not established
- Sequence and structural features of target almost certainly influential

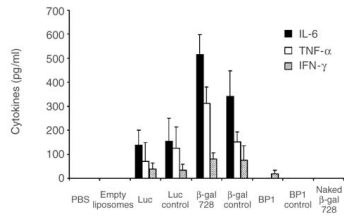
siRNA in practice: Off-target effects



1892-1897 | PNAS | February 17, 2004 | vol. 101 | no. 7

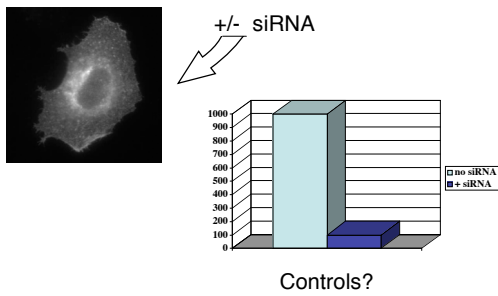
siRNA in practice: Non-specific effects

1. siRNAs encapsulated in liposomes
2. Injected into mice
3. Measured cytokines



NATURE BIOTECHNOLOGY VOLUME 23 NUMBER 4 APRIL 2005

Interpreting an siRNA experiment



Summary

RNAi biology

siRNA caveats

- Design
- Off-target
- Non-specific

Controls!!
