

Module 2: Expression Engineering

20.109

Lecture 3

October 18th, 2007

STEM Education

best way to achieve Adequate Yearly Progress?

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Excerpt from Medina, Jennifer. "Making Cash a Prize for High Scores on Advanced Placement Tests." *The New York Times*, October 15, 2007.

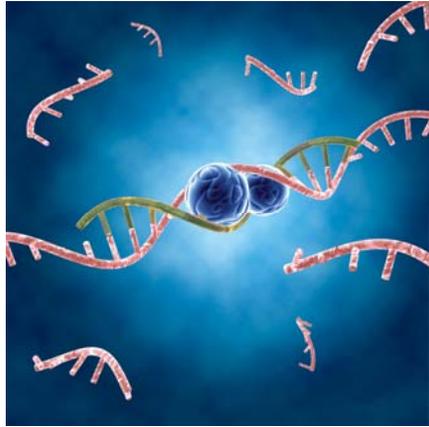
[<http://www.nytimes.com/2007/10/15/nyregion/15rewards.html>, accessed May 8, 2008.]

Excerpt from Kula, Adam and Alexandra Frean. "Make science easier, examiners are told." *The Times*, August 29, 2007. [<http://www.timesonline.co.uk/tol/news/uk/education/article2344054.ece>, accessed May 8, 2008.]

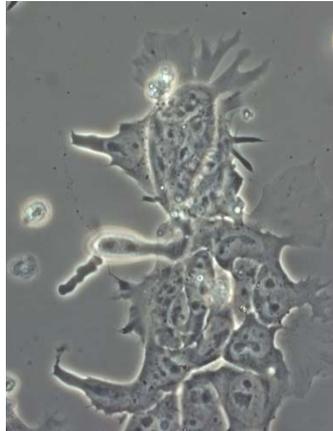
Excerpt from Cavanaugh, Sean. "Federal Rule Yields Hope for Science." *Education Week*, published online October 5, 2007.

Expression Engineering Experiment

Day 1

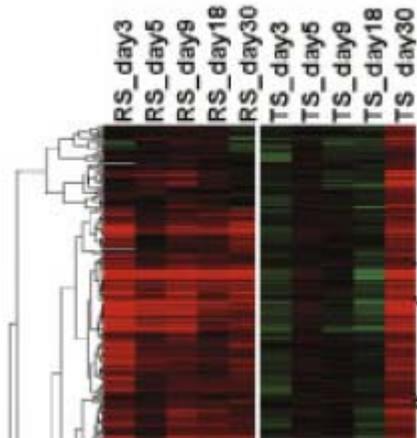


Day 2

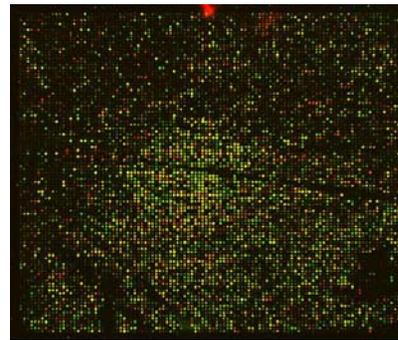


Day 3

Image of glowing luciferase in a microcentrifuge tube, removed due to copyright restrictions.



Day 6



Day 5

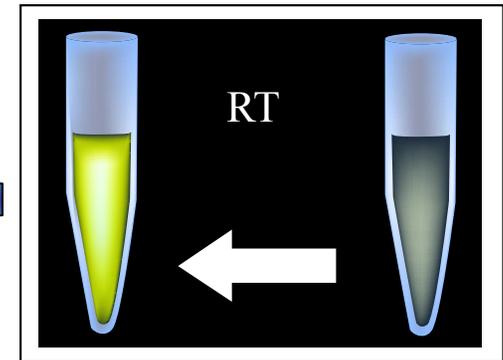


Figure by MIT OpenCourseWare.

Day 4

Image credits: Day 1 - Courtesy of Arkitek Studios. Used with permission. Day 2 - Courtesy of The Exploratorium. © The Exploratorium, <http://www.exploratorium.edu>. Day 4 - Figure by MIT OpenCourseWare. Day 5 - Dr. Natalie Kuldell. Day 6 - Courtesy of NIH.

Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (<http://ocw.mit.edu>), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].

Expression Engineering Experiment

Lecture 1

- intro to cell culture
- intro to gene exp'n/RNAi

Lecture 2

- transfection
- luciferase

Lecture 3

- off-target/nonspecific RNAi

Lecture 4

- Writing lecture
(Neal Lerner)

Lecture 5

- measuring gene express'n

Lecture 6

- microarray analysis
(Rebecca Fry)

Lecture 7

- high throughput technologies or
RNAi applications (no lab)

Lecture 8

- review of your data

RNAi

Operation or Battleship?

Images removed due to copyright restrictions.

Photos of the games "Operation" and "Battleship."

Where were you in 1998?

Content removed due to copyright restrictions.

News-related images:

- “France wins the World Cup”
- “Clinton under Investigation” [re: Monika Lewinski]
- President Clinton speaking at MIT commencement
- Movie poster for “Titanic”

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire^{*}, SiQun Xu^{*}, Mary K. Montgomery^{*}, Steven A. Kostas^{*†}, Samuel E. Driver[‡] & Craig C. Mello[‡]



Source: NIH.

Image removed due to copyright restrictions.

Excerpt from Table 1: Effects of sense, antisense and mixed RNAs on progeny of injected animals. In Fire, A., et al. "Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*." *Nature* 391 (19 February 1998). doi: 10.1038/35888.

small-RNA revolution

Image removed due to
copyright restrictions.
Cover of *Science* 298 no. 5602
(20 December 2002).
"New roles for RNAs."

of publications:

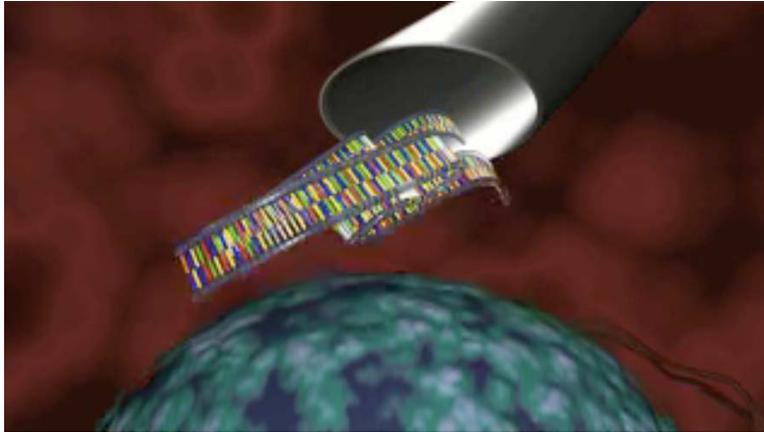
~15 in 1998

~1000 in 2000

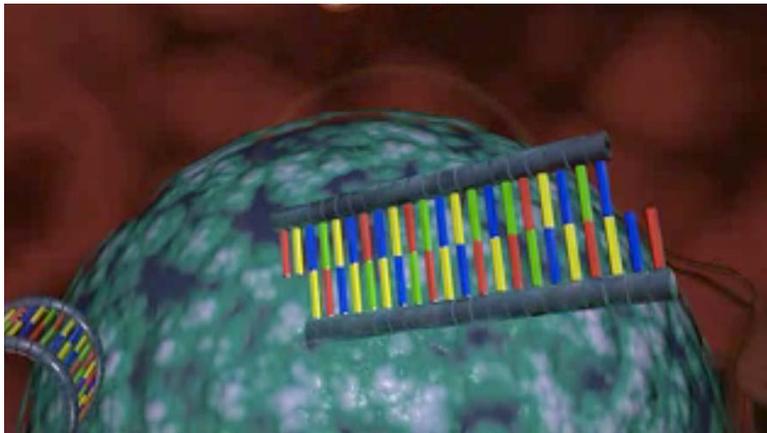
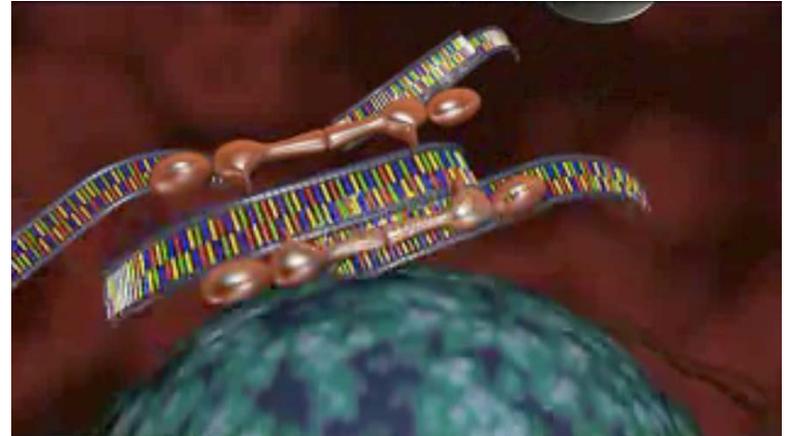
~ 4500 in 2007 (so far)

RNA interference vocabulary

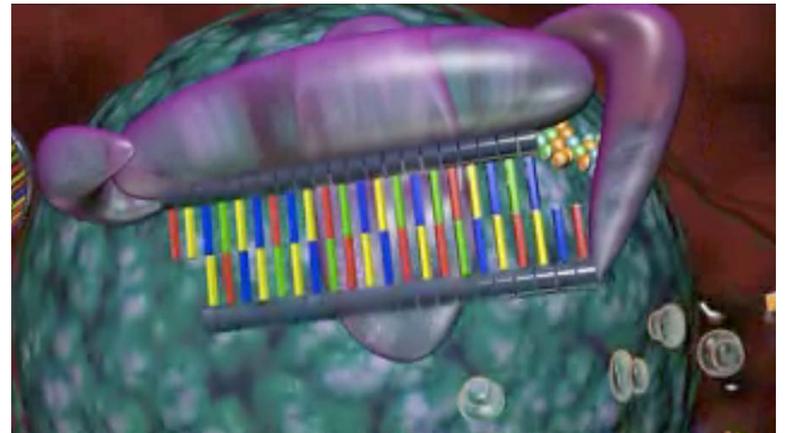
Transfection



Dicer



siRNA

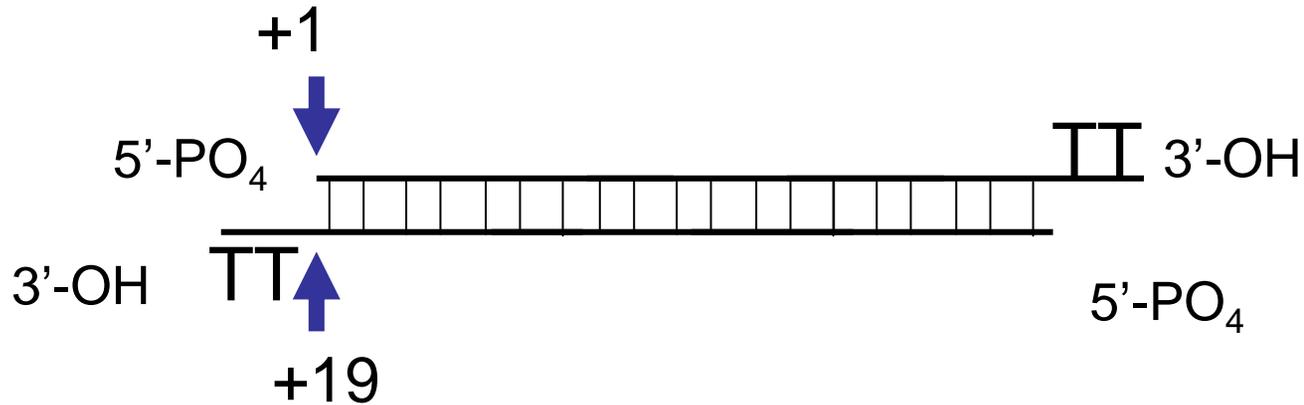


RISC

Courtesy of Arkitek Studios. Used with permission.

Source: RNAi Interface animation for nature.com (<http://www.nature.com/focus/rnai/animations/index.html>)

Nomenclature for siRNAs



RNA

AUG

UAA

“sense”

DNA

ATG

TAA

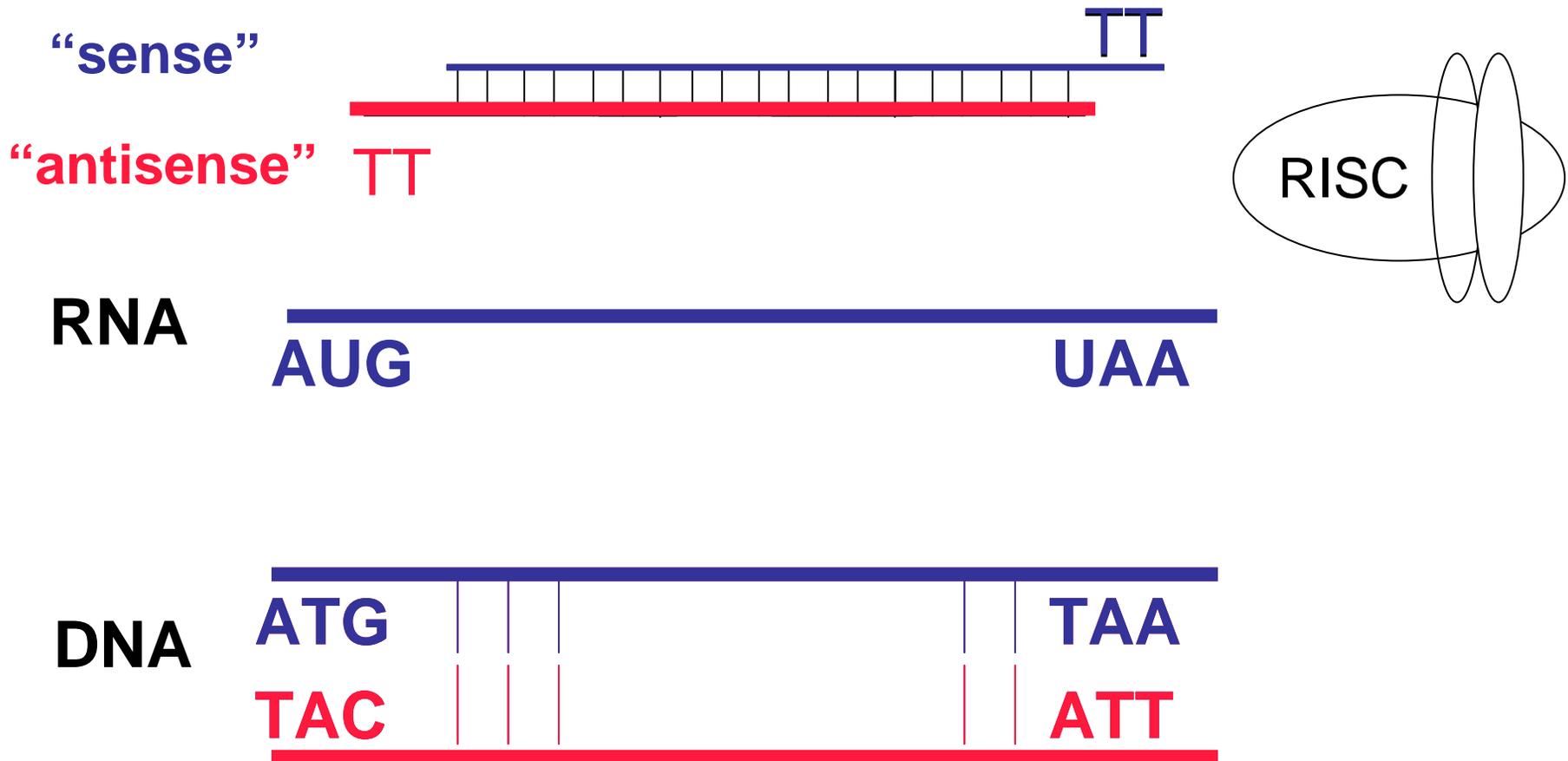
template

TAC

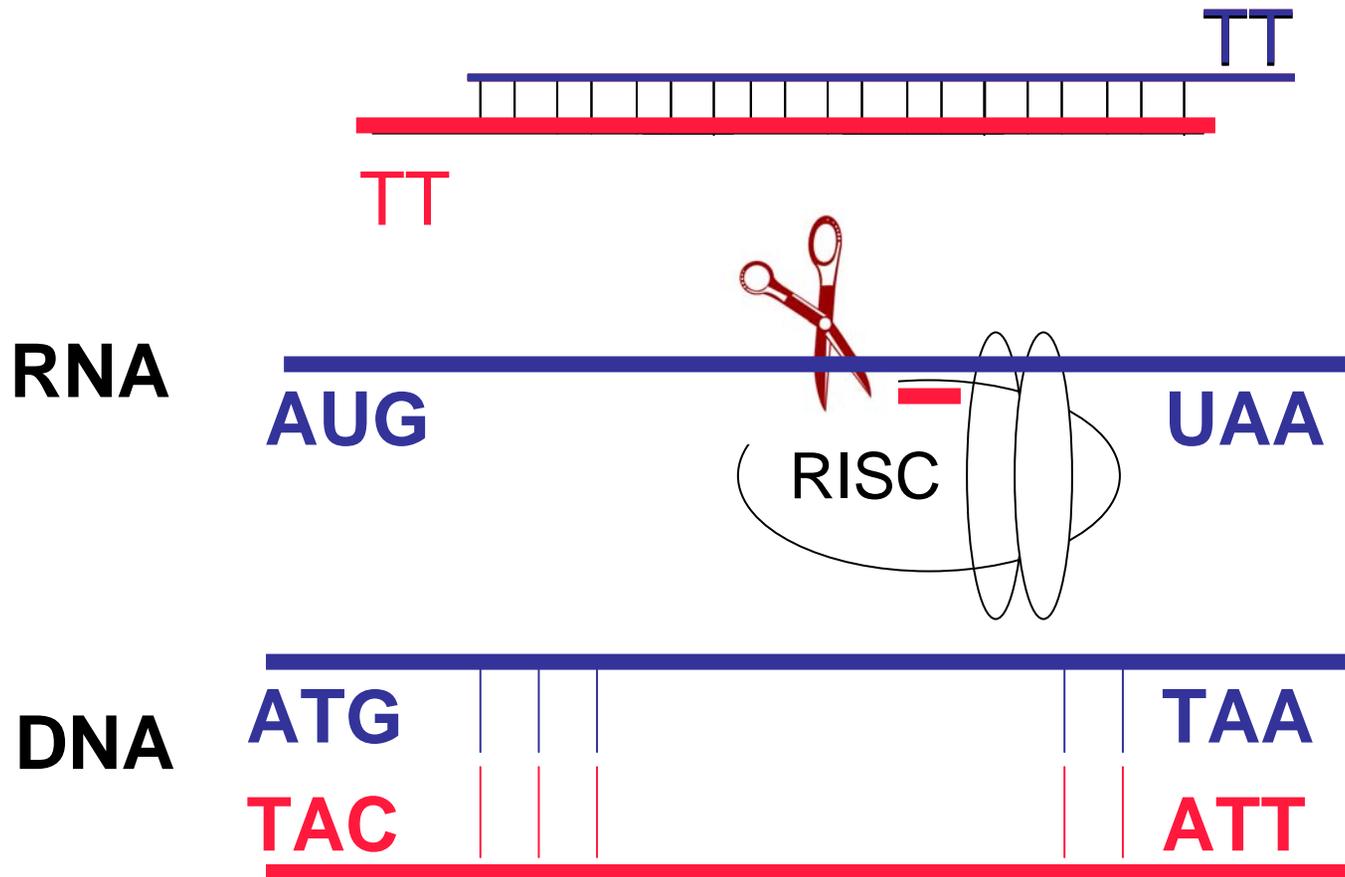
ATT

“coding strand” “template strand”

Nomenclature for siRNAs



Nomenclature for siRNAs



Delivery vs expression of dsRNA

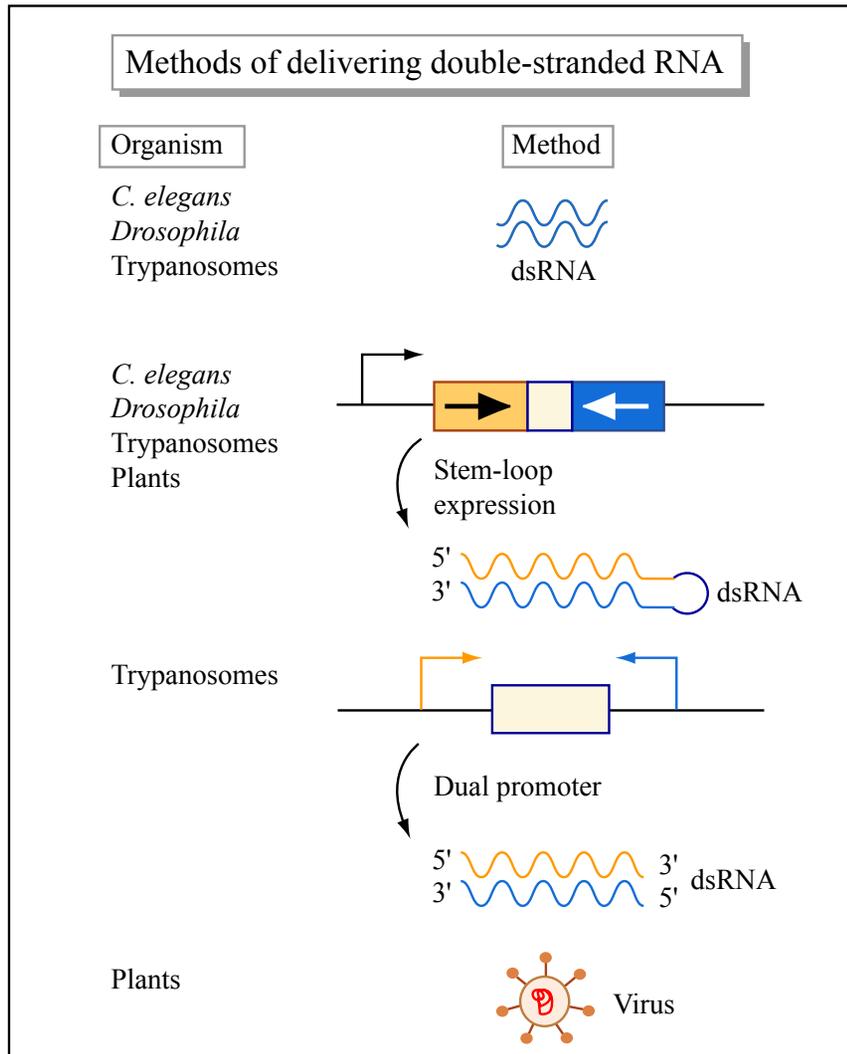


Figure by MIT OpenCourseWare.

See “Post-transcriptional gene silencing by dsRNA.”
 Nature Reviews Genetics 2001; 2:110-119.

RNAi: siRNA vs miRNA

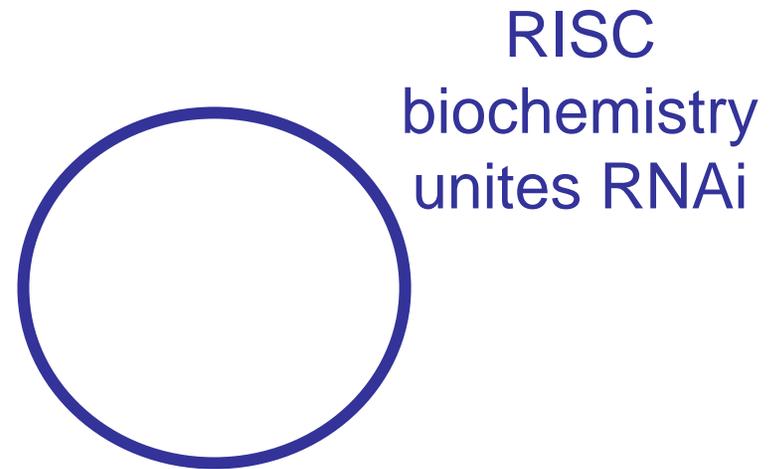


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fig from Svoboda review in Curr Opin Mol Ther. 2007 9(3):248

RNAi (on paper)

Highly effective

>90% reduction in gene expression

Highly specific

only targeted mRNA gets degraded

Reproducible

effects same with each expt'l repeat

Image removed due to
copyright restrictions.

Photo of the game "Operation."

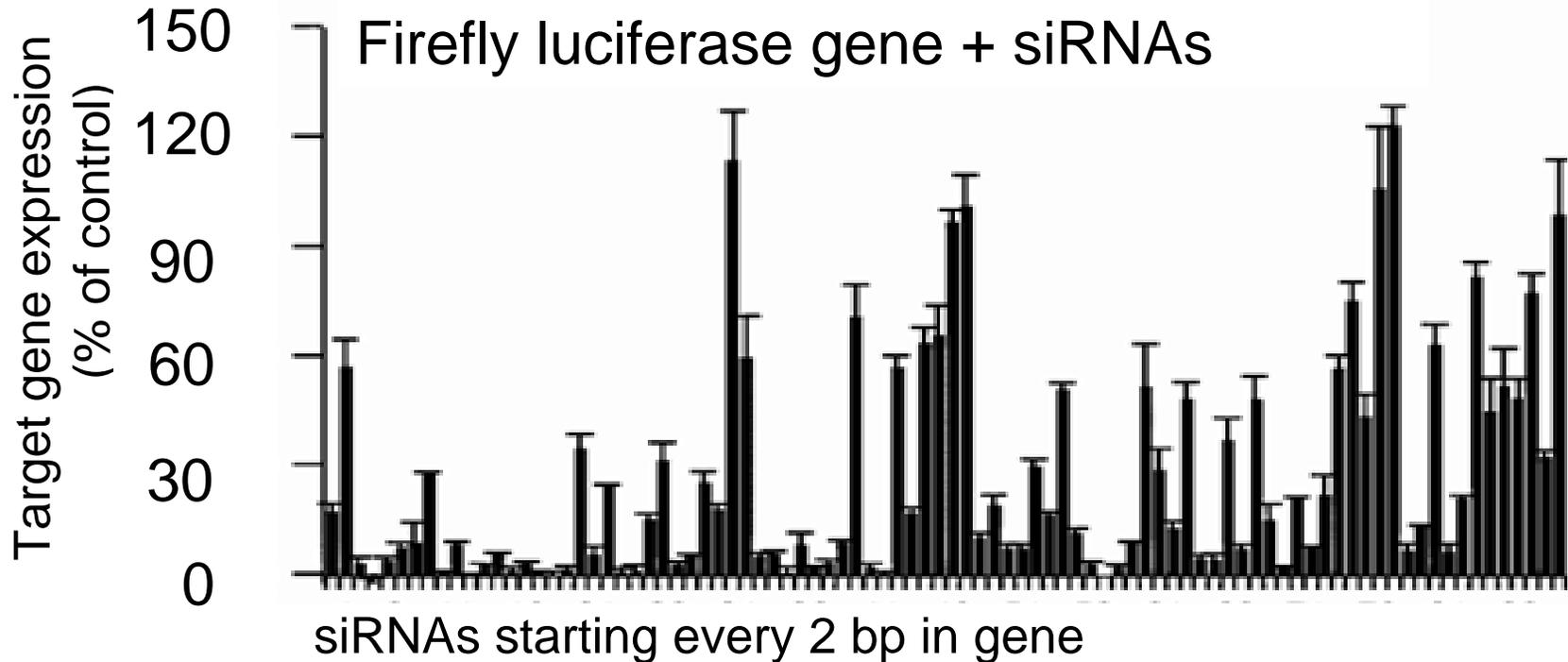
And wouldn't it also be great if it
was genetically stable and
applicable in many cell types

siRNA in practice

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Photo of the game "Battleship."

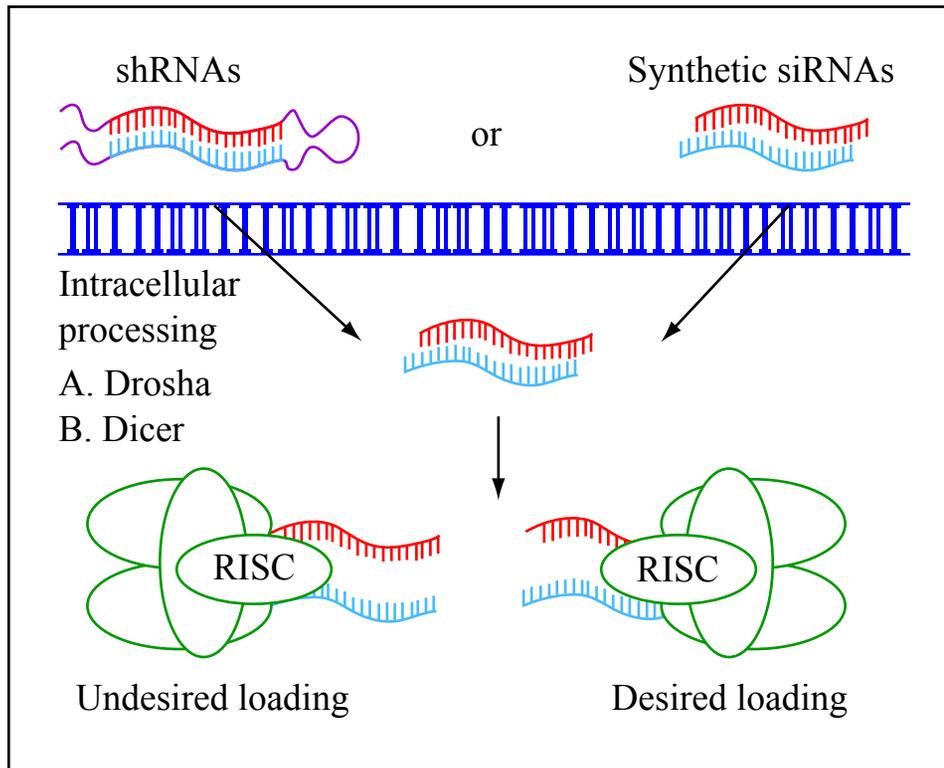
Design ambiguities



Courtesy of Anastasia Khvorova. Used with permission.

Reynolds, A., et al. "Rational siRNA design for RNA interference." *Nature Biotechnology* 22, 326 - 330 (2004). doi:10.1038/nbt936.

siRNA: design rules



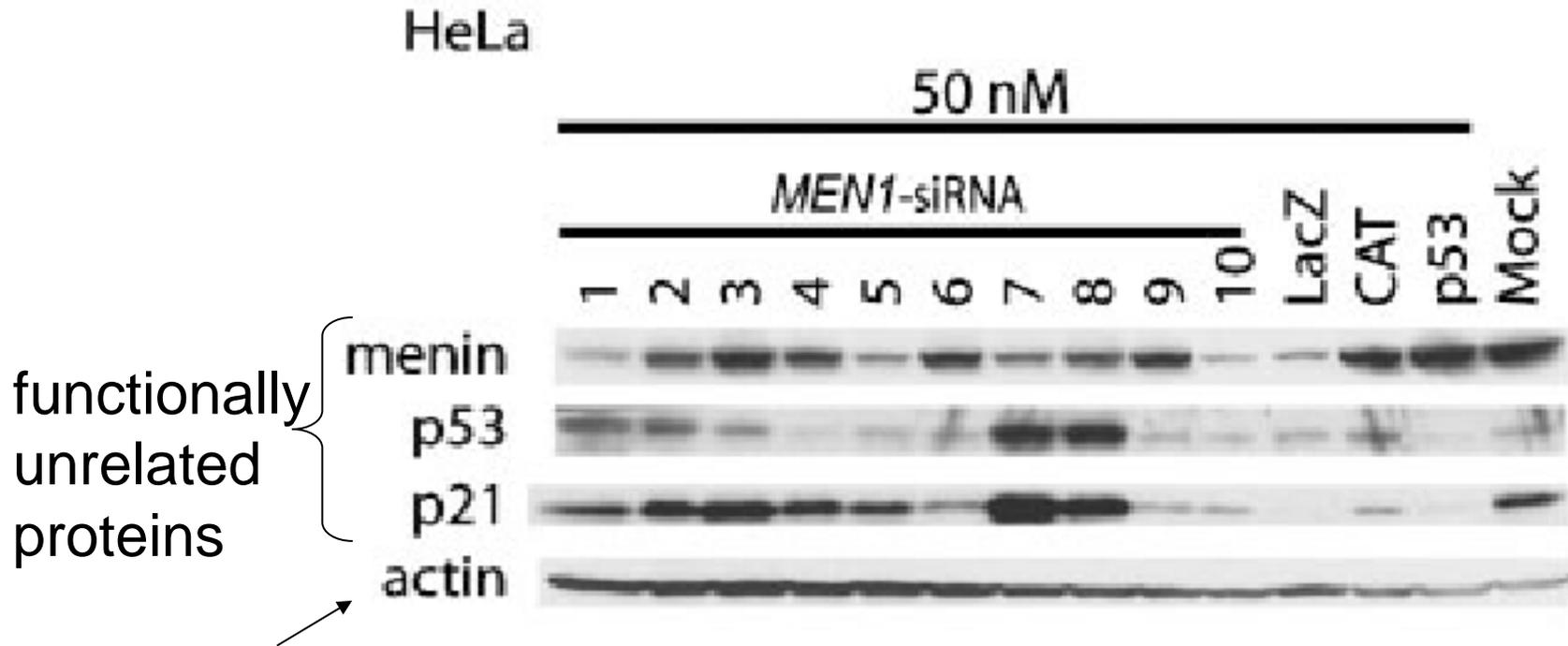
- ✓ 5' to 3' unwinding: easier end loads into RISC
- ✓ A at position 3 on sense strand
- ✓ not G or C at position 19 on sense strand

Figure by MIT OpenCourseWare.

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

siRNA in practice: Off-target effects

Western blot



Some siRNAs directed against menin decrease p53 and p21

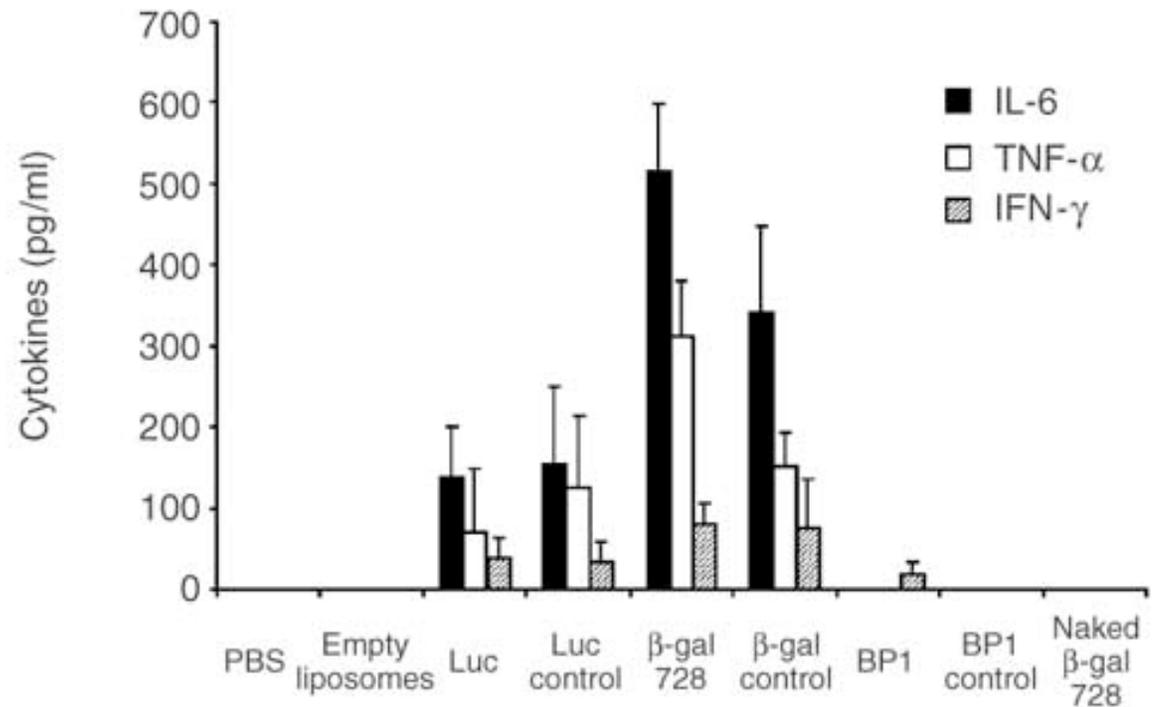
Courtesy of National Academy of Sciences, U. S. A. Used with permission.

Source: Scacheri, P. C., et al. "Short interfering RNAs can induce unexpected and divergent changes in the levels of untargeted proteins in mammalian cells." *PNAS* 101 no. 7 (February 17, 2004): 1892-1897. DOI:10.1073/pnas.0308698100.

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siRNA: Non-specific effects

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



Courtesy of Ian MacLachlan. Used with permission.

Judge, A. D., et al. "Sequence-dependent stimulation of the mammalian innate immune response by synthetic siRNA." *Nature Biotechnology* 23 no. 4 (April 2005): 457-462. doi:10.1038/nbt1081.

Interpreting an siRNA experiment

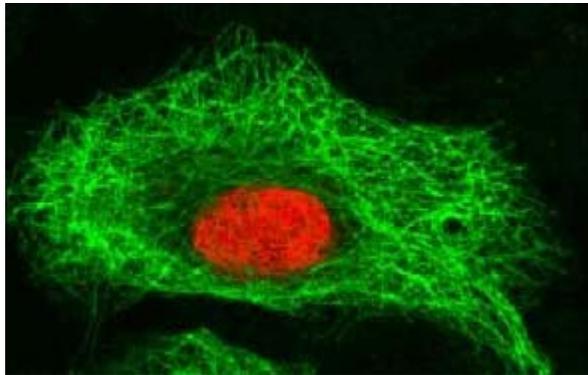
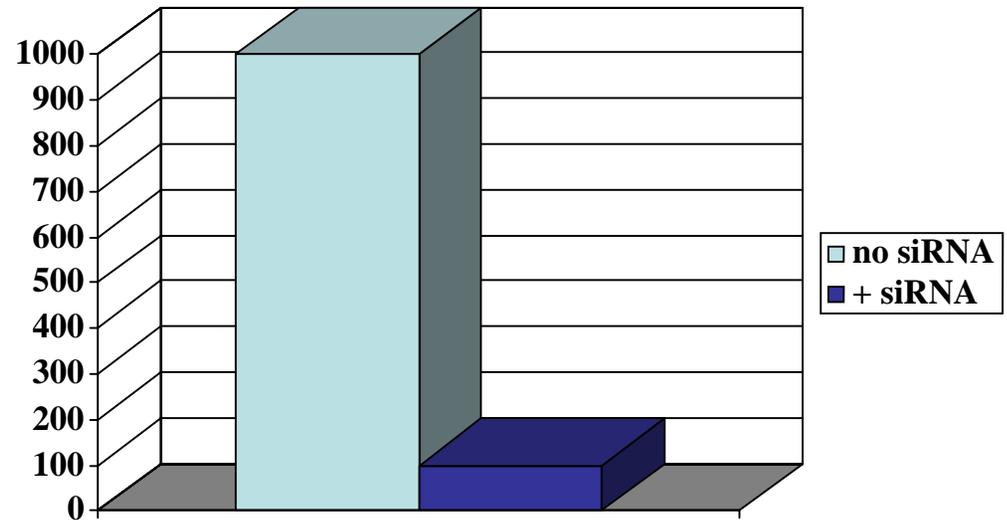
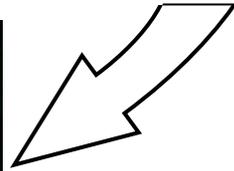


Image courtesy of NOAA.

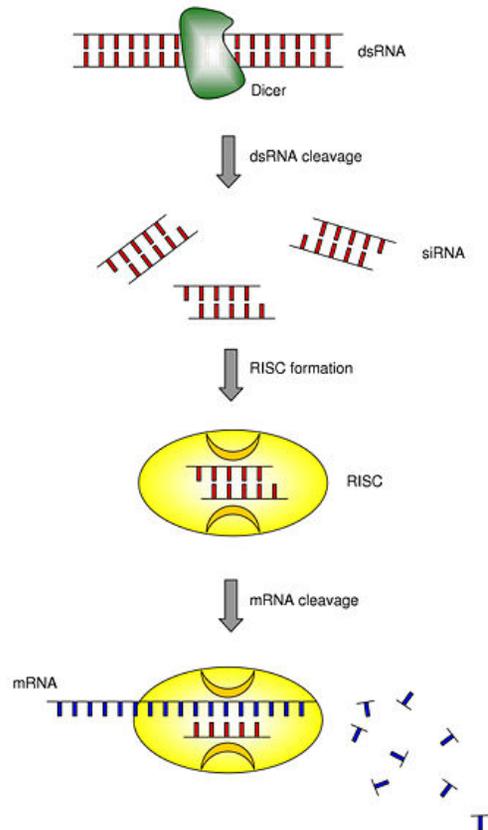
+/- siRNA



Controls?

Summary

RNAi biology



Courtesy of Simone Mocellin and Maurizio Provenzano. *J Trans Med* 2 (2004): 39.
doi: 10.1186/1479-5876-2-39.

Image removed due to copyright restrictions.
Photo of the game "Operation."

siRNA caveats



Photo courtesy of [Colby Palmer](#).

- Design
- Off-target
- Non-specific

Controls!!

Image removed due to copyright restrictions.
Photo of the game "Battleship."

the end