

Module 2: Expression Engineering

20.109

Lecture 2

October 16th, 2007

Public Understanding?

Image removed due to copyright restrictions.

From “The DNA Diet,” AKC Gazette, April 2006:

“Scientists are seeking the recipes for dog foods that can attack illness at the genetic level”

[http:// www.ardenmoore.com/pets/dna-diet.html](http://www.ardenmoore.com/pets/dna-diet.html)

Public Understanding?

Images removed due to copyright restrictions.

Website screenshots from <http://www.thednadiet.com>, advertising a service using DNA testing to develop a personal diet plan.

Public Understanding?

Images removed due to copyright restrictions.

Photo of anti-GMO protesters from <http://www.gmfreeireland.org>

Cartoon of “mad scientist” character

Editorial cartoon about fast-track bill to allow use of genetically modified food ingredients without labeling

Public Understanding?

Image removed due to
copyright restrictions.

Cartoon of “mad scientist”
character.

Text removed due to copyright restrictions.

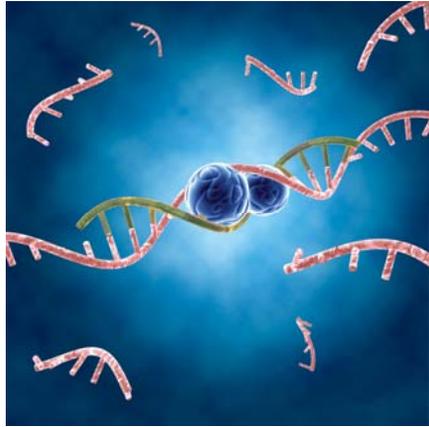
Excerpt from "Lawmakers, Administration Clash over Science Funding."

by Aliya Sternstein. *National Journal's Technology Daily*, February 14, 2007.

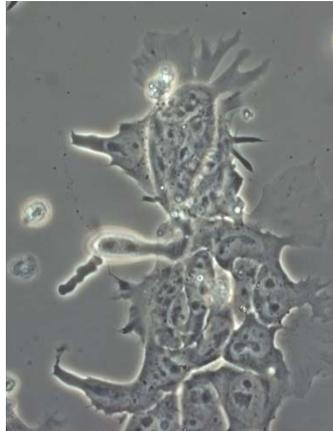
http://www.govexec.com/story_page.cfm?articleid=36137&ref=relink (accessed May 7, 2008)

Expression Engineering Experiment

Day 1

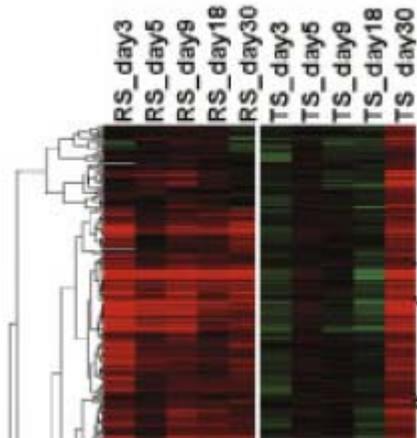


Day 2

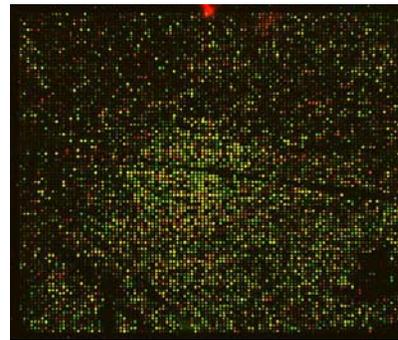


Day 3

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



Day 6



Day 5

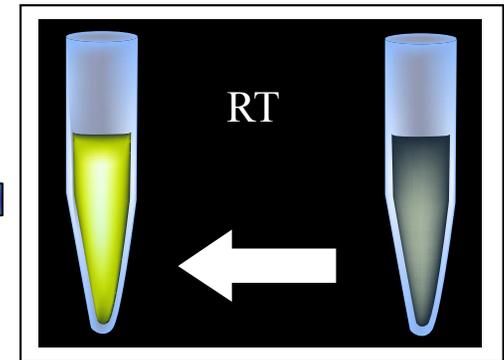


Figure by MIT OpenCourseWare.

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

DNA on the move: nature's ways

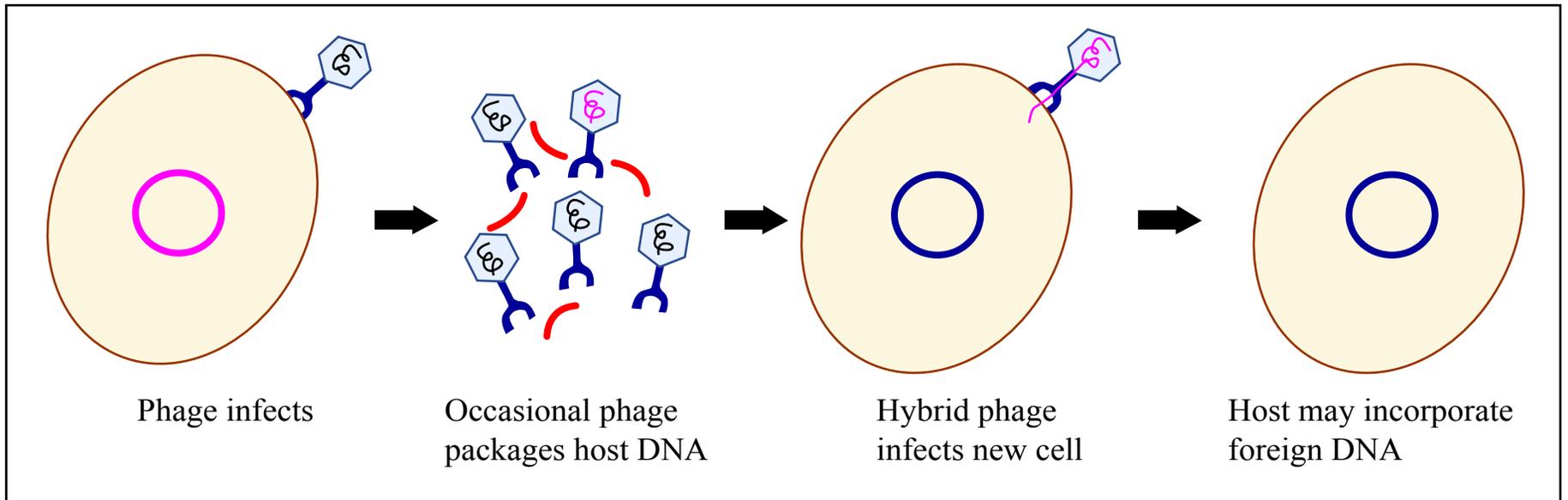


Figure by MIT OpenCourseWare.

DNA on the move: nature's ways

1. Viral transduction

Text and image removed due to copyright restrictions.
Excerpt from: Pearson, Helen. "Ancient Human Virus Resurrected."
Nature (31 October 2006). DOI:10.1038/news061030-4.

[http://blogs.nature.com/news/
blog/2006/10/ancient_human_
virus_resurrecte.html](http://blogs.nature.com/news/blog/2006/10/ancient_human_virus_resurrecte.html)

DNA on the move: nature's ways

1. Viral transduction
2. Conjugation

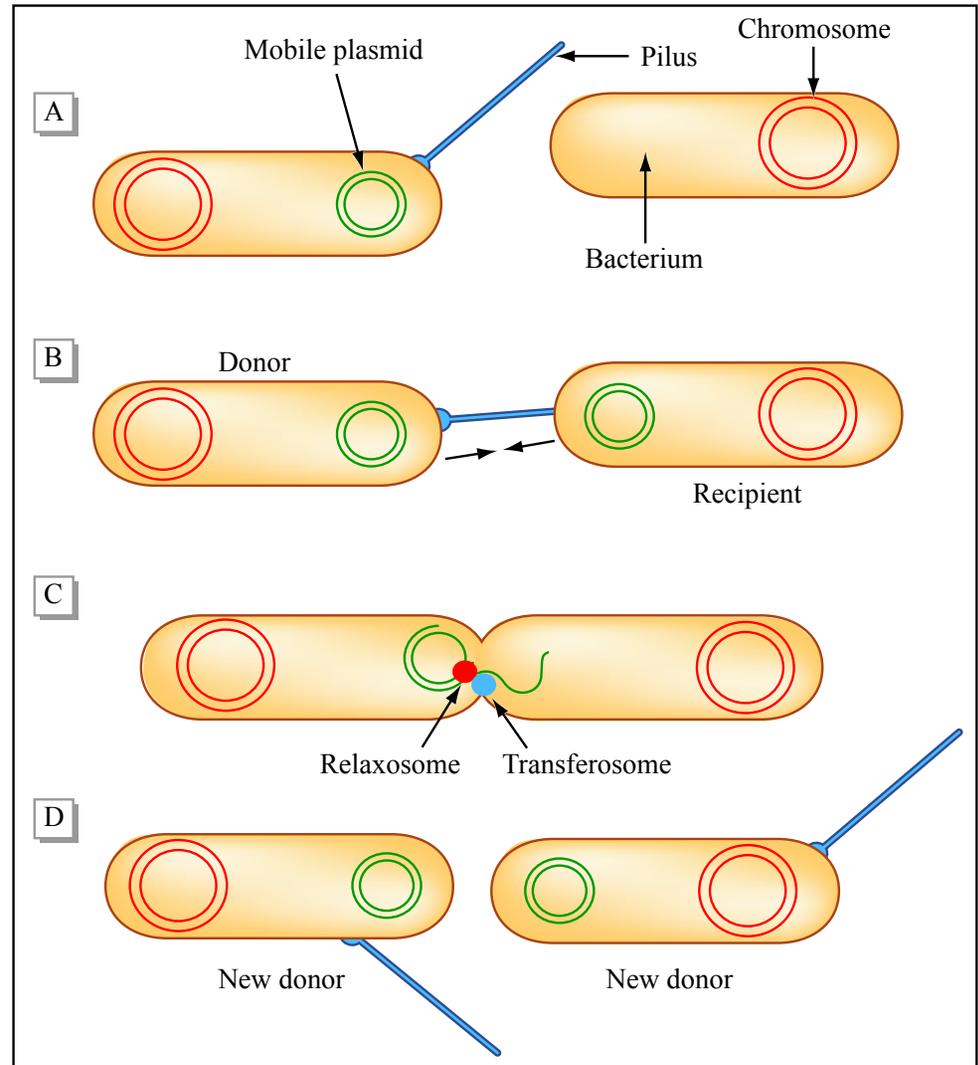


Figure by MIT OpenCourseWare.

DNA on the move: nature's ways

1. Viral transduction

2. Conjugation

3. Transformation

TABLE 1. Properties of natural genetic competence in four well-studied organisms

Species	Habitat	DNA uptake	Regulation
<i>Streptococcus pneumoniae</i>	Human upper respiratory tract	Nonspecific	Cell-cell signaling; inhibited in stationary phase
<i>Bacillus subtilis</i>	Soil	Nonspecific	Cell-cell signaling; various nutritional controls
<i>Neisseria gonorrhoeae</i>	Human genital tract	Sequence-specific	Constitutive
<i>Haemophilus influenzae</i>	Human upper respiratory tract	Sequence-specific	Induced by starvation; requires increased intracellular cAMP

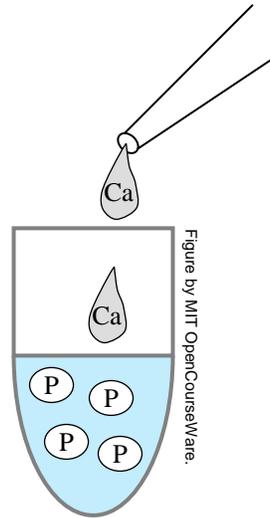
S. pneumoniae and *B. subtilis* are Gram positive, *N. gonorrhoeae* and *H. influenzae* are Gram negative. Natural genetic competence has also been reported in many other genera³, including *Acinetobacter*, *Helicobacter*, *Methylobacterium*, *Micrococcus*, *Mycobacterium*, *Pseudomonas* and *Streptomyces*.

Courtesy Elsevier, Inc., <http://www.sciencedirect.com>. Used with permission.

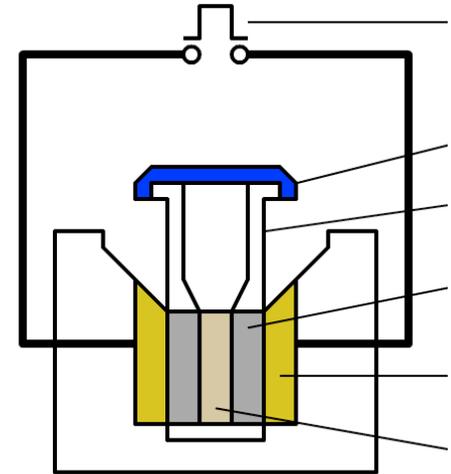
Jonathan M. Solomon and Alan D. Grossman "Who's competent and when: regulation of natural genetic competence in bacteria" Trends in Genetics (1996) 12(4):150

DNA on the move: **in the lab**

1. Viral transduction
2. Conjugation
- 3. Transformation**



chemical

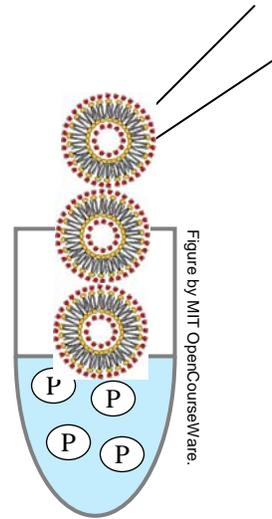


electrical

Courtesy of [Zephyris](#).

DNA on the move: **in the lab**

1. Viral transduction
2. Conjugation
- 3. Transformation**

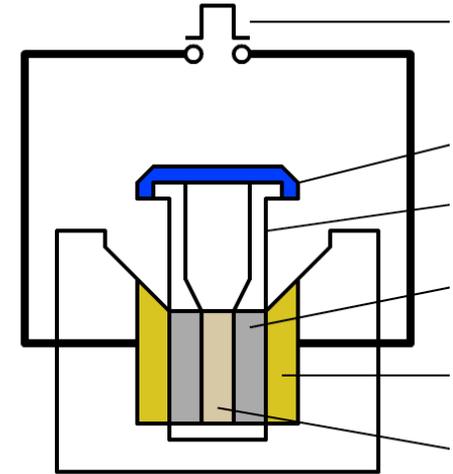


chemical

microinjection

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See http://firstivf.net/laboratory_tour.htm.



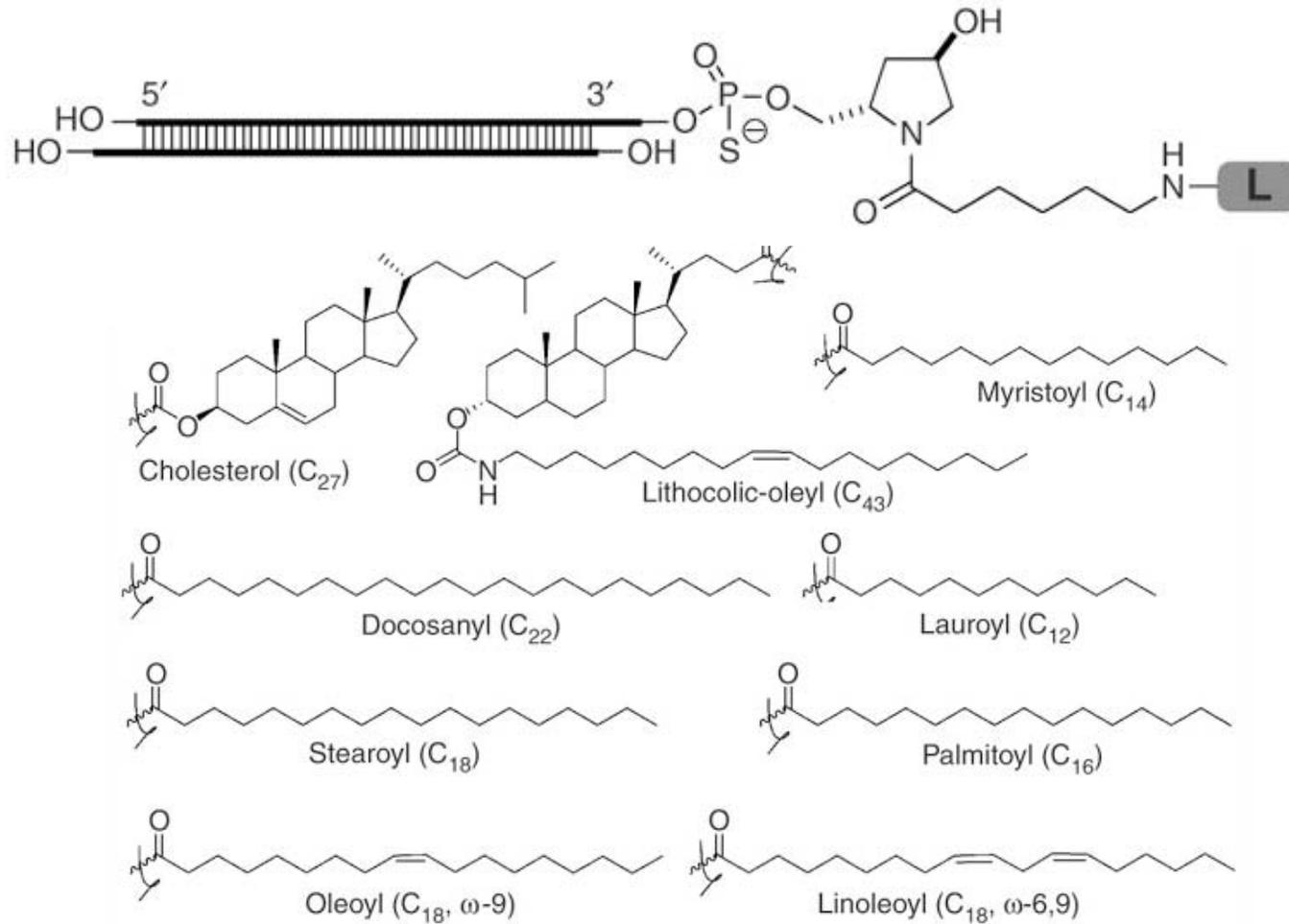
electrical

Courtesy of [Zephyris](#).

biolistics

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DNA on the move: RNAi delivery

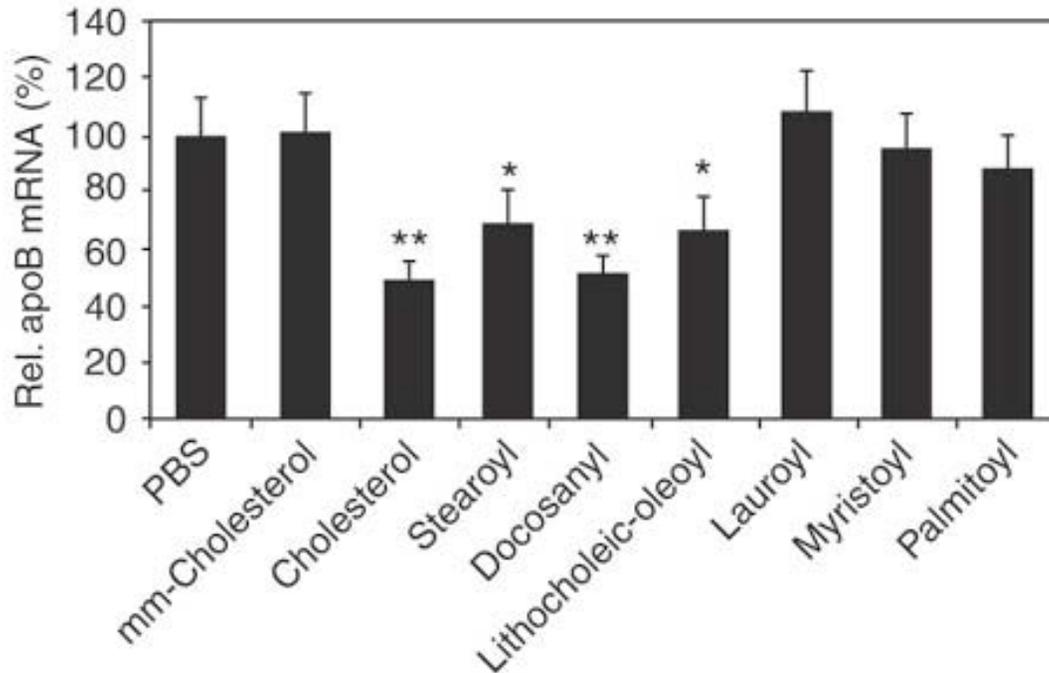
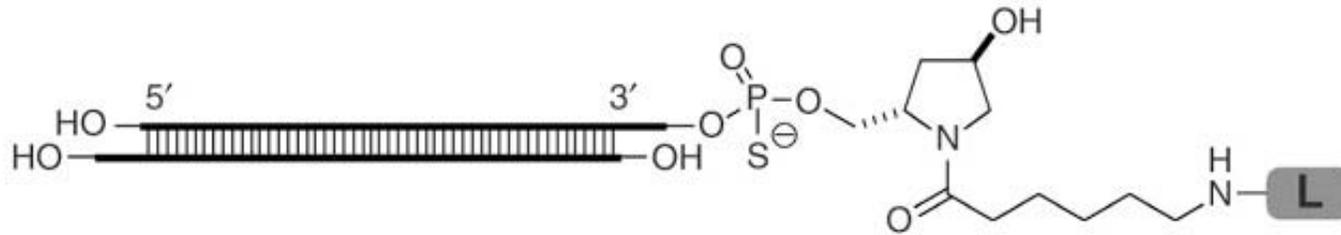


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Nature Biotechnology (2007) 25: 1149 - 1157

Mechanisms and optimization of in vivo delivery of lipophilic siRNAs

DNA on the move: RNAi delivery



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Nature Biotechnology (2007) 25: 1149 - 1157

Mechanisms and optimization of in vivo delivery of lipophilic siRNAs

DNA on the move: RNAi delivery

SCIENCE FOR POLICYMAKERS

THE SCIENCE OF BIODEFENSE: RNAI

Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science
Volume 5, Number 2, 2007

DNA on the move: RNAi delivery

SCIENCE FOR POLICYMAKERS

THE SCIENCE OF BIODEFENSE: RNAI

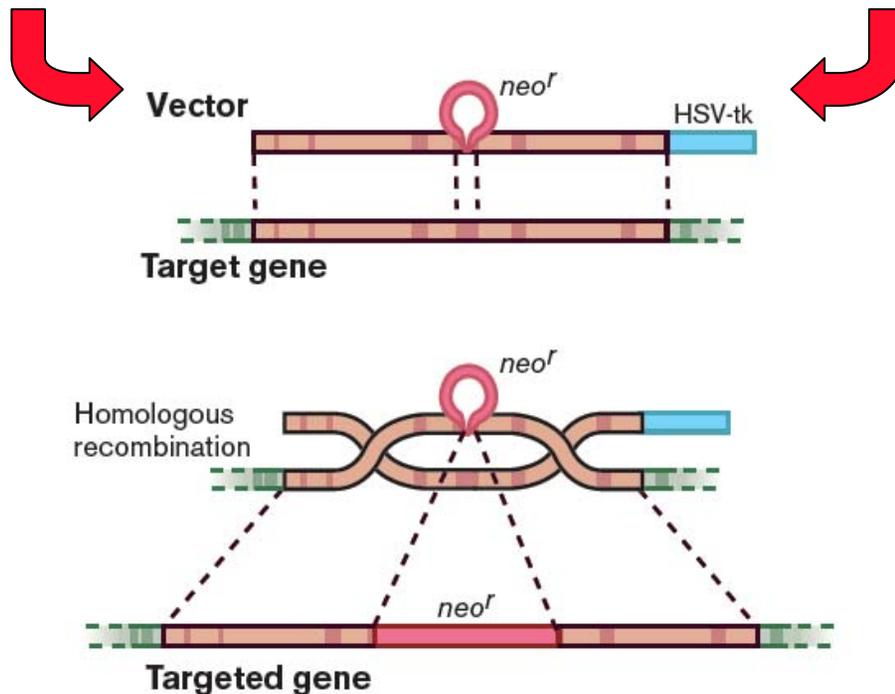
Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science
Volume 5, Number 2, 2007

While there are great potential clinical and countermeasure benefits to RNAi research, it also has dual-use potential. RNAi was described in a 2006 Institute of Medicine report, *Globalization, Biosecurity, and the Future of the Life Sciences*, as a technology that “could also be used to manipulate gene expression with the intent to do harm.”⁶ For example, if RNAi-based aerosol therapies prove effective, that same technology could be used to create an aerosolized RNAi-based weapon. The UK suggested such a scenario in their report to the Biological Weapons Convention in 2006: an RNAi weapon that could silence genes integral to our immune systems.⁷ The perpetrators of such an attack could render victims defenseless against infection by even normally innocuous microbes.

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DNA on the move: transgenic animals

Photos of the laureates removed due to copyright restrictions.
Mario R Capecchi, Sir Martin J. Evans, and Oliver Smithies.



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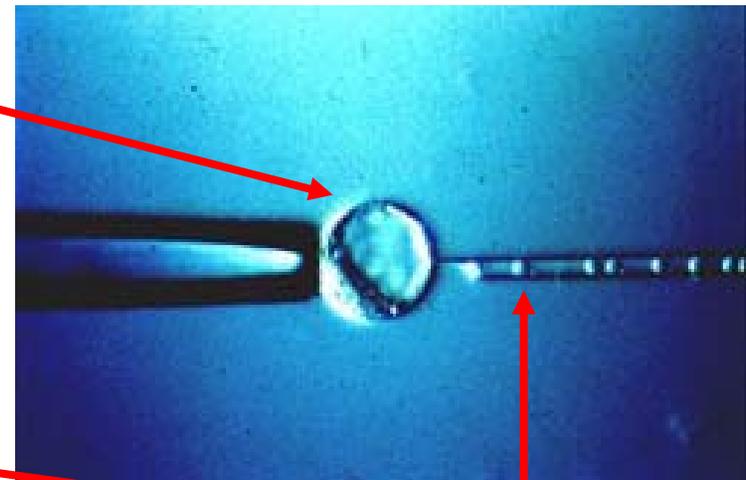
Cite as: Natalie Kuldell. Course materials for 20.109 Laboratory Fundamentals in Biological Engineering, Fall 2007. MIT OpenCourseWare (<http://ocw.mit.edu>), Massachusetts Institute of Technology. Downloaded on [DD Month YYYY].

DNA on the move: **transgenic animals**

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Mario R Capecchi, Sir Martin J. Evans, and Oliver Smithies.



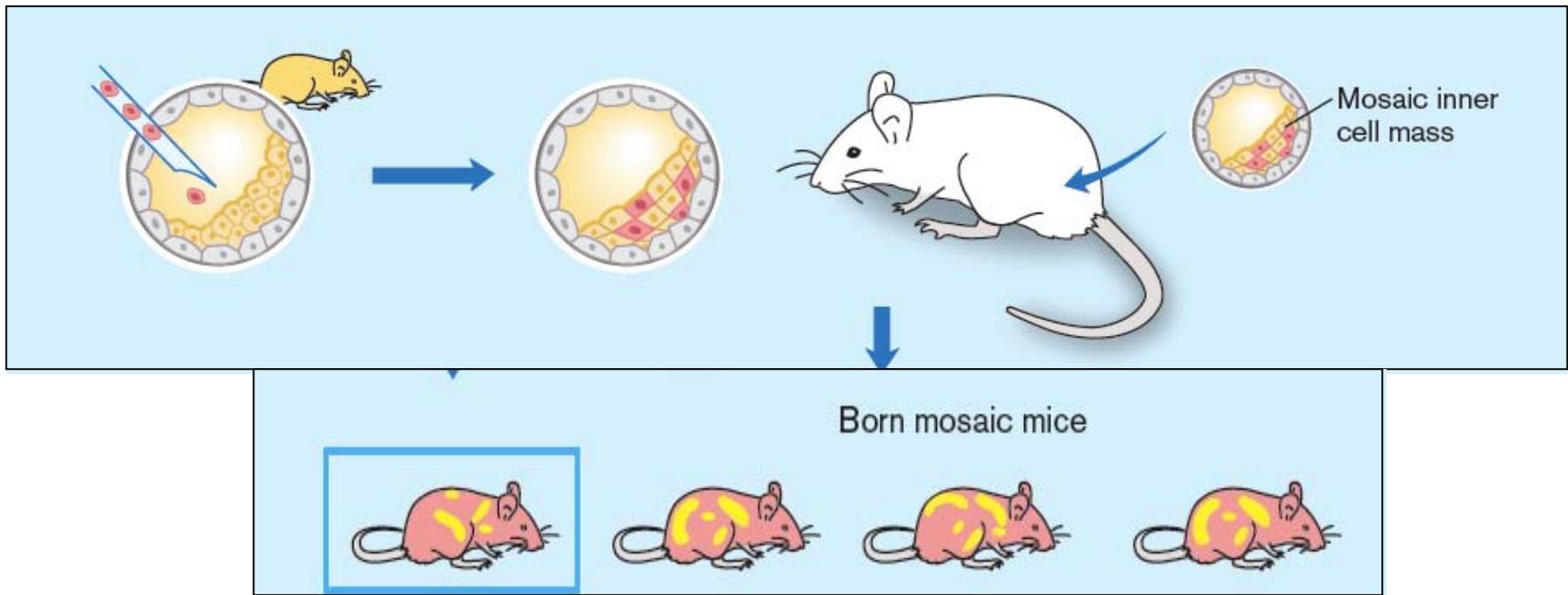
Photo of mice removed due to copyright restrictions.



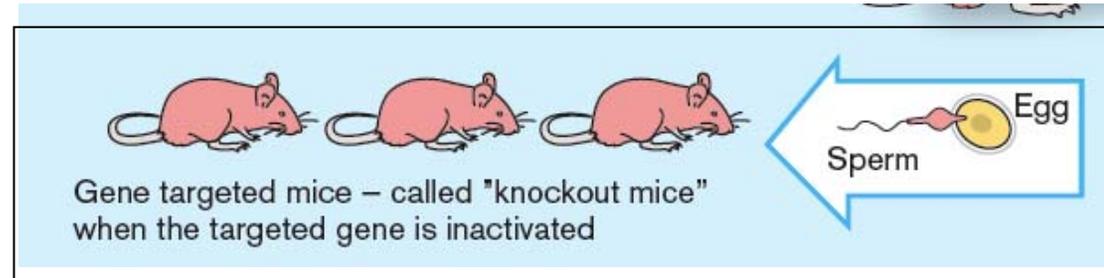
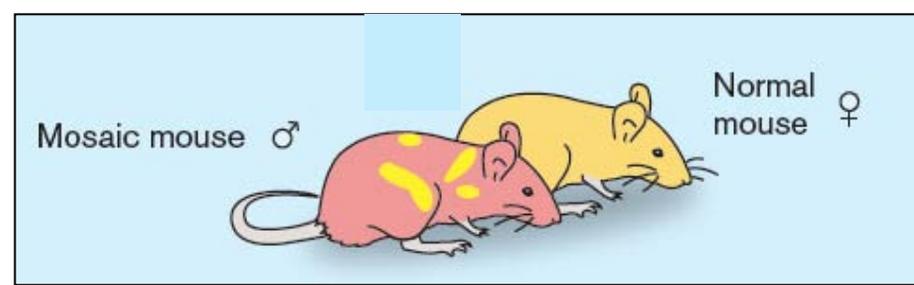
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DNA on the move: transgenic animals

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Mario R Capecchi, Sir Martin J. Evans, and Oliver Smithies.



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Photo of mice removed due to copyright restrictions.

An advertisement for genOway. The top half features a close-up profile of a white mouse drinking from a glass water bottle. The background is a gradient of blue. Text on the left side reads: 'Provider in customized and ready-to-use genetically modified mouse and rat models. Knock-out, conditional Knock-out, Knock-in, RNAi, humanized, transgenic mice and rats'. At the bottom, the genOway logo is on the left and the tagline 'Reliable & Innovative Solutions for Transgenesis' is on the right.

Courtesy of genOway. Used with permission.

DNA on the move: transgenic animals

Photo of "Alba, the fluorescent bunny,"
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See <http://www.ekac.org/gfpbunny.html#gfpbunnyanchor>.

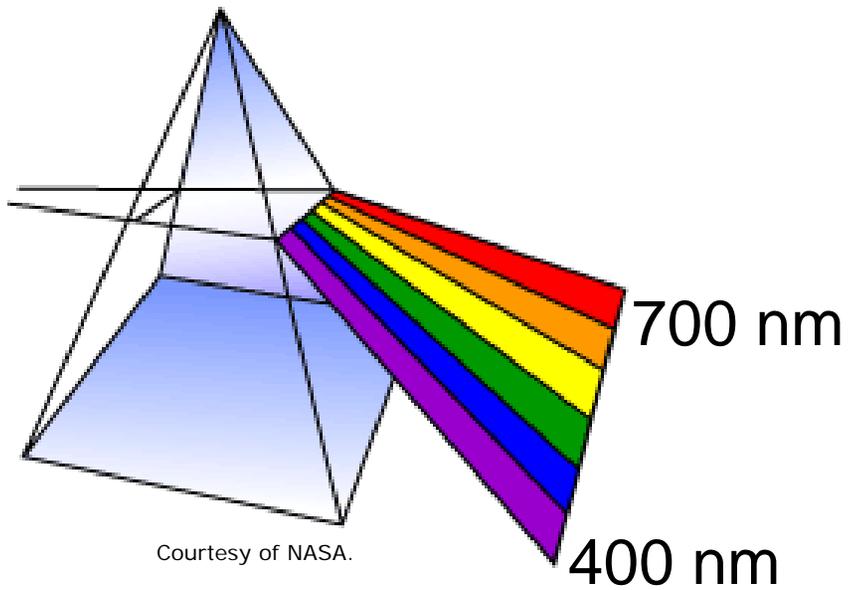


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Courtesy of NOAA.
2007: Exploring the Inner Space of the Celebes Sea



Courtesy of NASA.

Fluorescence

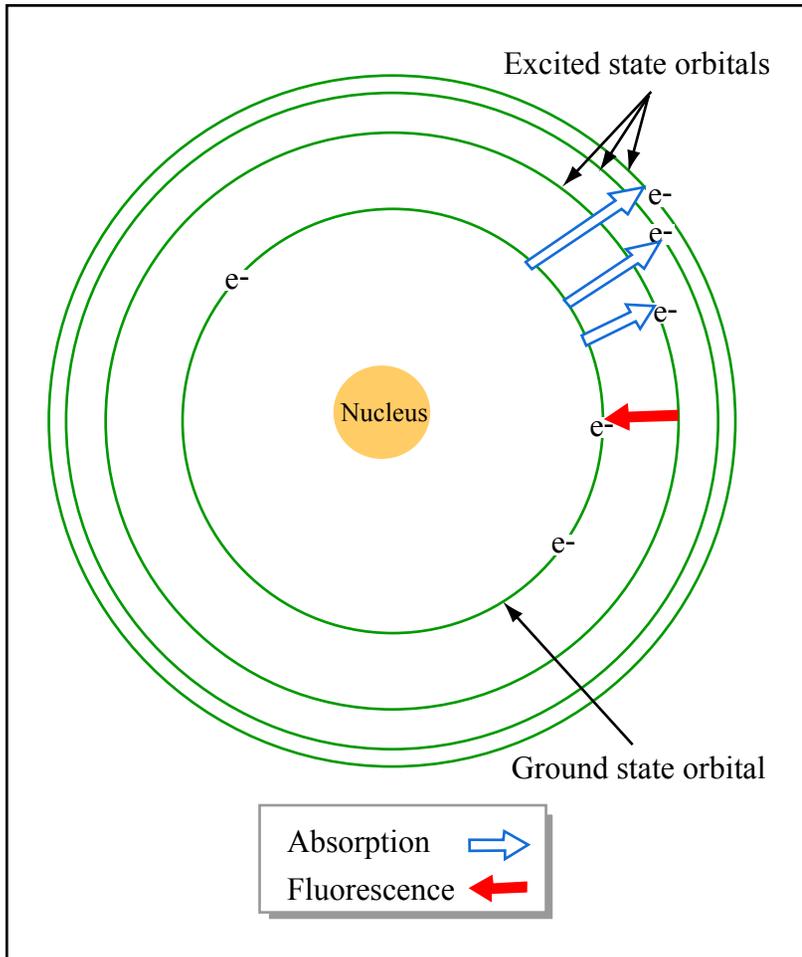
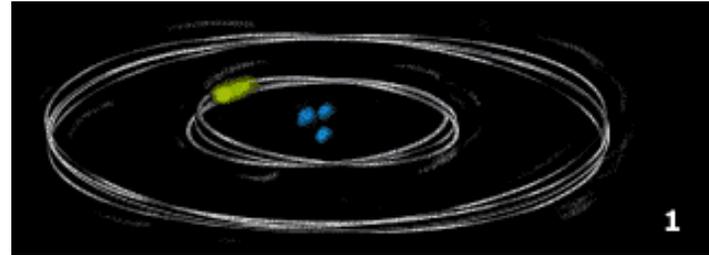


Figure by MIT OpenCourseWare.

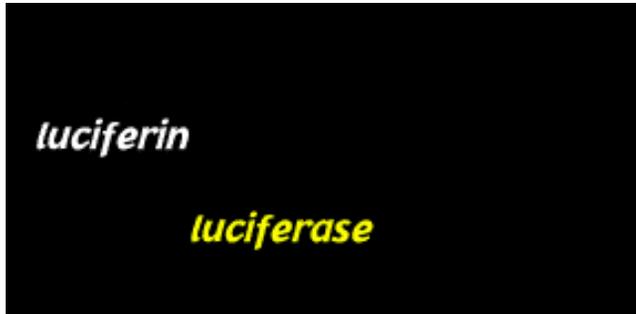


Courtesy of Steven Haddock. Used with permission.
Source: Haddock, S.H.D., C. M. McDougall, J. F. Case.
"The Bioluminescence Web Page." <http://lifesci.ucsb.edu/~biolum/>
(created 1997; updated 2007; accessed Fall 2007).



Courtesy of NOAA.
2007: Exploring the Inner Space of the Celebes Sea

Bioluminescence



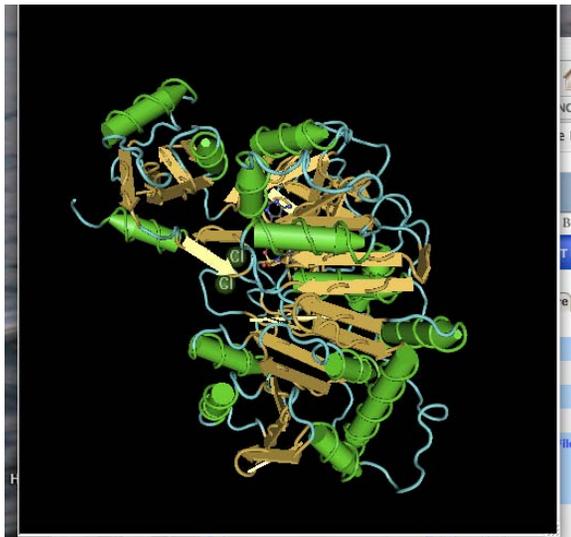
Chemical reaction diagrams removed due to copyright restrictions.
See Figures 1, 2, and 4 in:

<http://www.chemistryexplained.com/Ar-Bo/Bioluminescence.html>

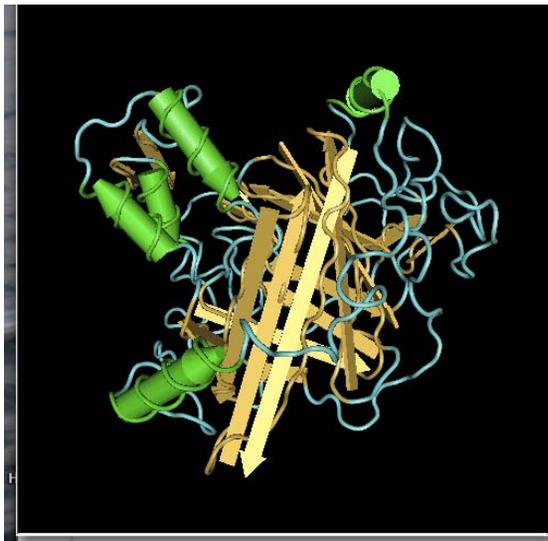
<http://www.lifesci.ucsb.edu/~biolum/chem/>

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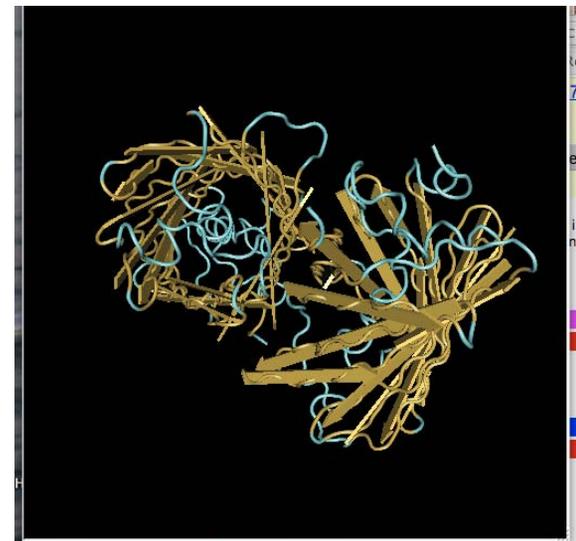
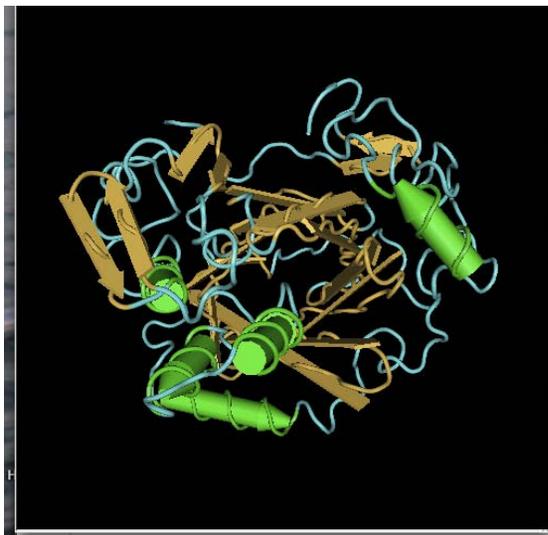
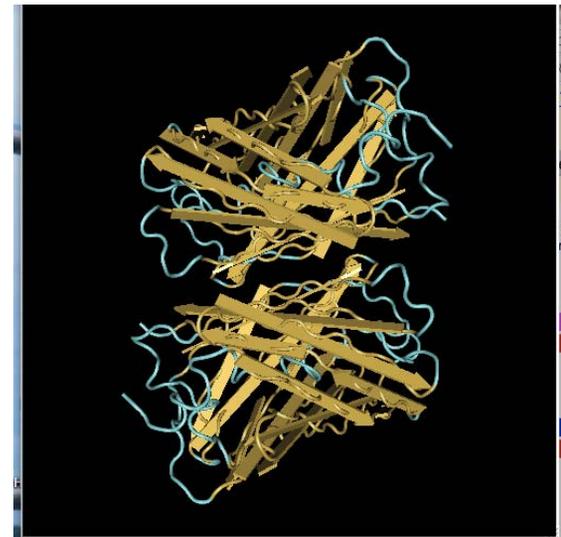
firefly luciferase



renilla luciferase



GFP



Images generated in Cn3D
(<http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml>)

BLAST 2 Sequences

Photinus pyralis luciferase mRNA

Renilla reniformis luciferase mRNA

 **Blast 2 Sequences results**

PubMed Entrez **BLAST** OMIM Taxonomy Structure

BLAST 2 SEQUENCES RESULTS VERSION BLASTN 2.2.17 [Aug-26-2007]

Match: Mismatch: gap open: gap extension:
x_dropoff: expect: wordsize: [Filter](#) View option
Masking character option Masking color option
 Show CDS translation

Sequence 1: lcl|1
Length = 1644

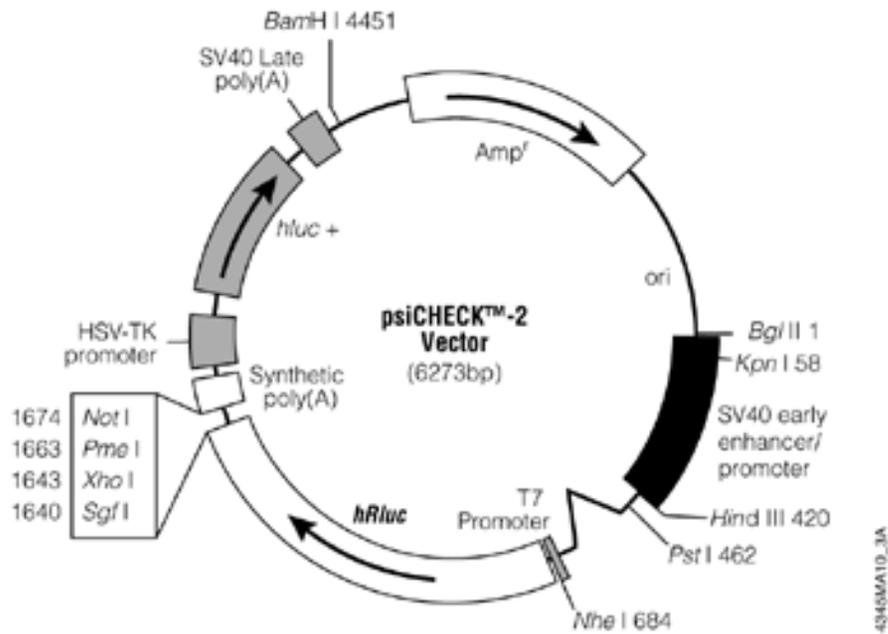
Sequence 2: lcl|65536
Length = 1195

No significant similarity was found

CPU time: 0.10 user secs. 0.07 sys. secs 0.17 total secs.

<http://www.ncbi.nlm.nih.gov/BLAST/bl2seq/wblast2.cgi>

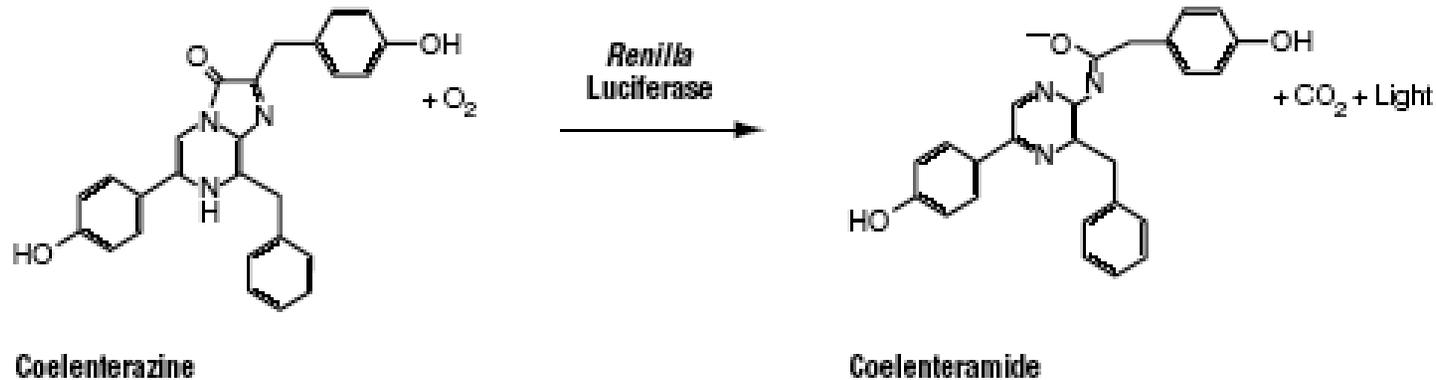
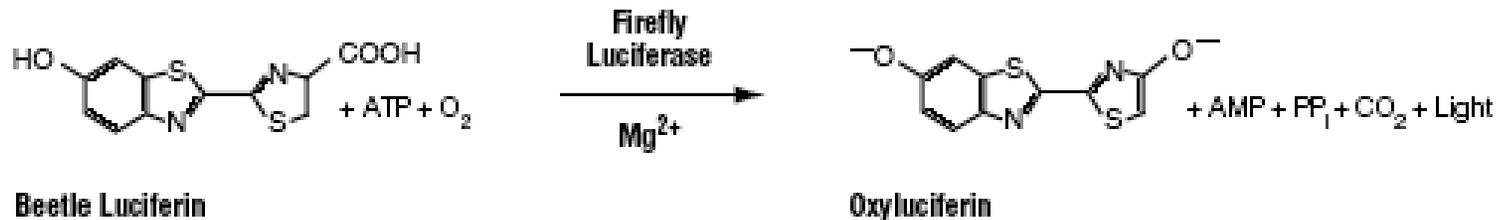
Bioluminescence in the lab



Promega psiCHECK2

Courtesy of Promega Corporation. Used with permission.

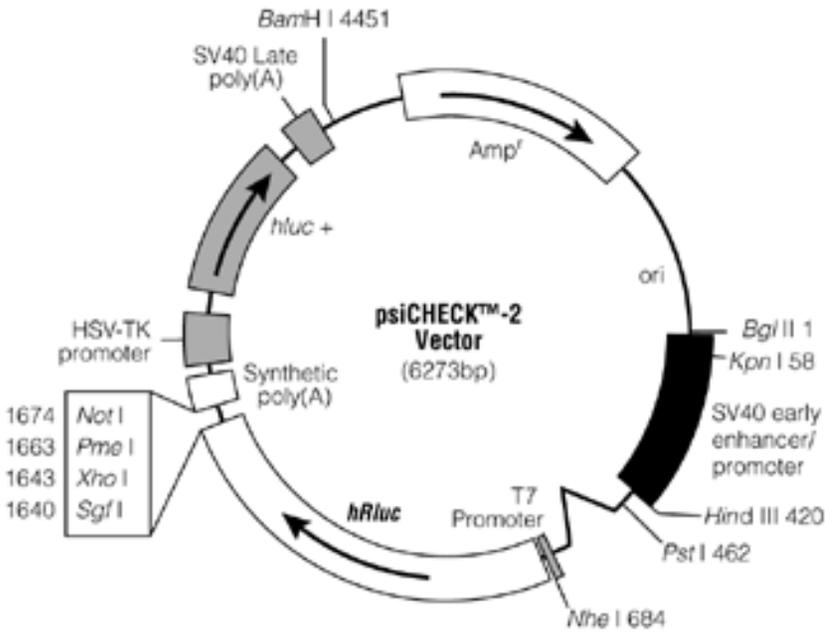
Two bioluminescent reactions



Promega online information, Dual-Glo luciferase assay

Courtesy of Promega Corporation. Used with permission.

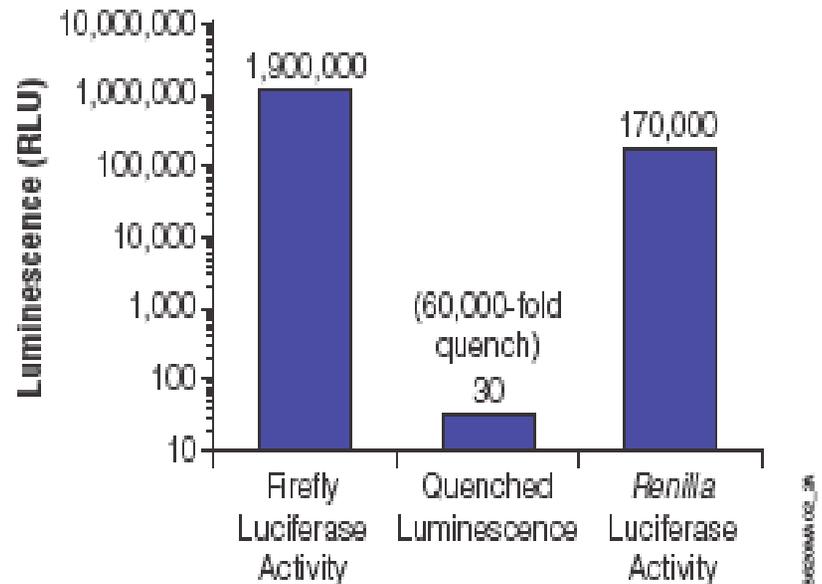
Bioluminescence in the lab



Promega psiCHECK2

Courtesy of Promega Corporation. Used with permission.

See “quenching” of rxn with purified luciferase proteins



Courtesy of Promega Corporation. Used with permission.

It's not easy being green

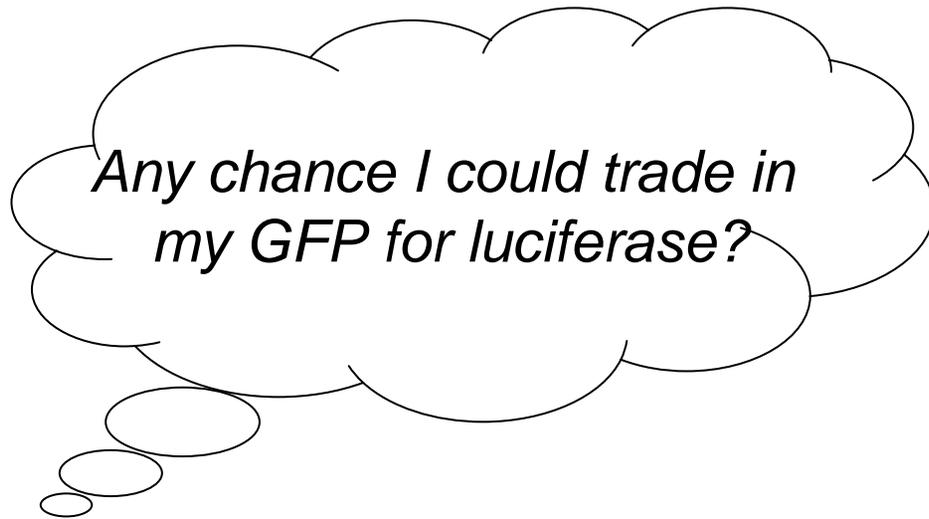


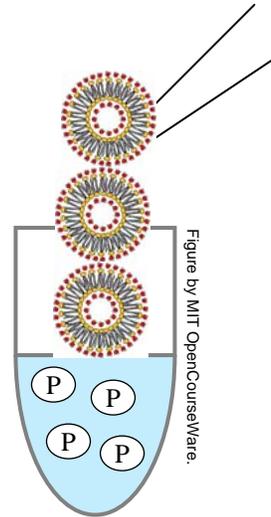
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See <http://www.ekac.org/gfpbunny.html#gfpbunnyanchor>.

"Mad scientist" cartoons removed due to copyright restrictions.

Summary

1. DNA on the move

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RNAi delivery
transgenic animals

2. Let there be light



Courtesy of NOAA.
2007: Exploring the Inner Space of the Celebes Sea



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