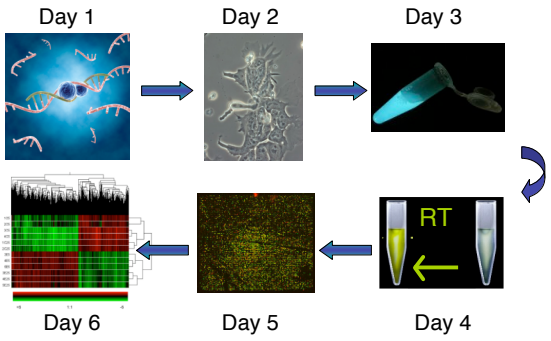
Module 2: Expression Engineering

20.109
Lecture 2
October 16th, 2007

Expression Engineering Experiment

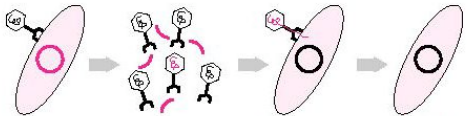


Expression Engineering Experiment

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

1. Viral transduction



phage infects

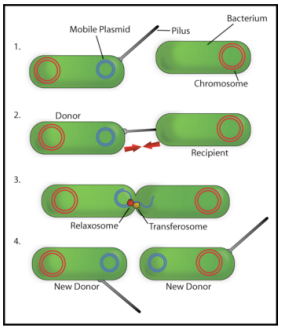
occasional phage packages host DNA

hybrid phage infects new cell

host may incorporate foreign DNA

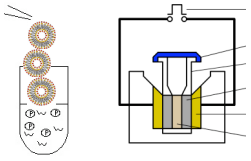
DNA on the move: nature's ways

1. Viral transduction
2. Conjugation



DNA on the move: in the lab

1. Viral transduction
2. Conjugation
3. Transformation

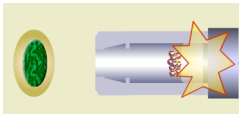


chemical

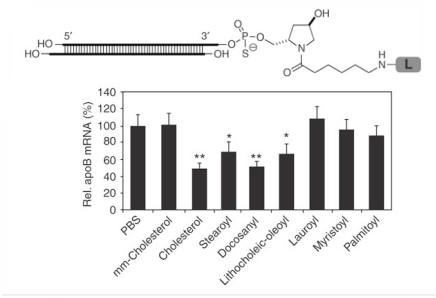
electrical

microinjection

biolistics

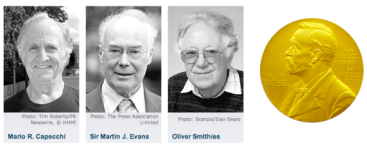


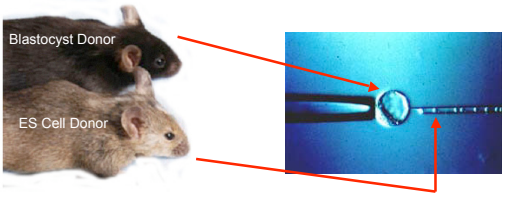
DNA on the move: RNAi delivery

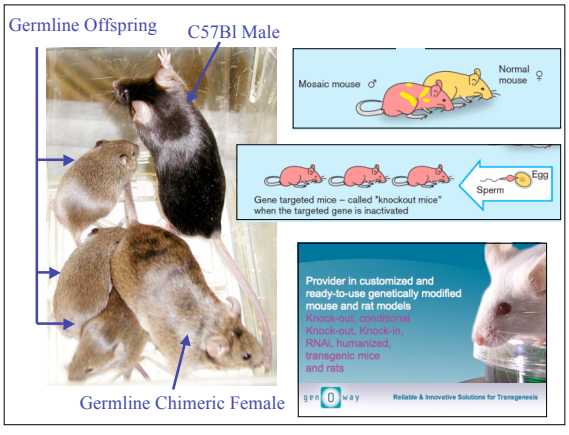


Nature Biotechnology (2007) 25: 1149 - 1157
Mechanisms and optimization of in vivo delivery of lipophilic siRNAs

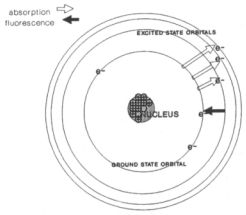
DNA on the move: transgenic animals

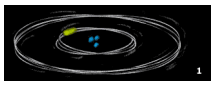


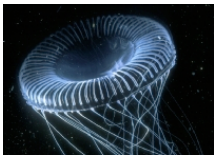




Fluorescence





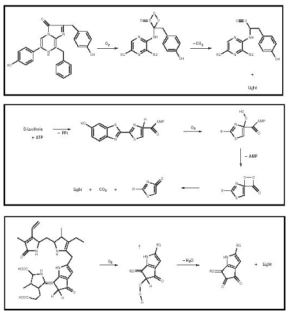


http://www.lifesci.ucsb.edu/~biolum/chem/

Bioluminescence

luciferin

luciferase



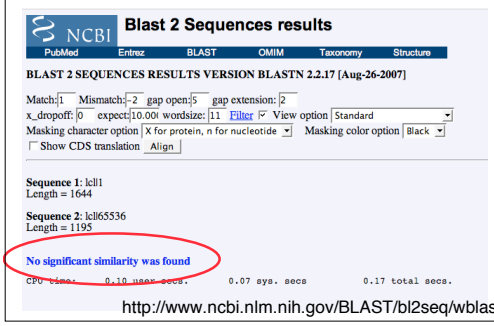
http://www.lifesci.ucsb.edu/~biolum/chem/

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

BLAST 2 Sequences

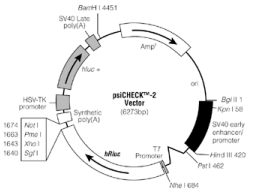
Photinus pyralis luciferase mRNA

Renilla reniformis luciferase mRNA



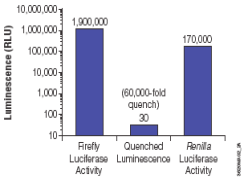
http://www.ncbi.nlm.nih.gov/BLAST/bl2seq/wblast2.cgi

Bioluminescence in the lab



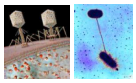
Promega psiCHECK2

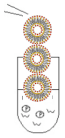
See "quenching" of rxn with purified luciferase proteins



Summary

1. DNA on the move





RNAi delivery

transgenic animals

2. Let there be light