

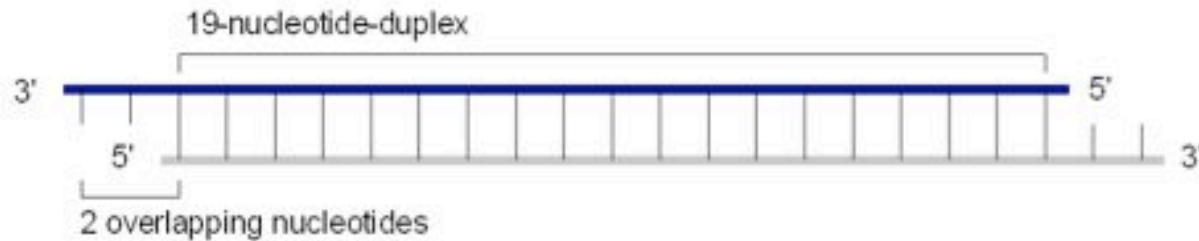
- Announcements
- Pre-lab Lecture
  - ❖ Mod 2 overview
  - ❖ siRNA basics
  - ❖ Tissue culture
  - ❖ Today in Lab (Mod 2 Day 1)

# Announcements

- Introducing... Steve, TA for Module 2
- Module 2 heads-up
  - Journal club presentations next week
  - No lecture on Day 3 *↳ write a post for all*
  - Day 4 lecture will be Mod 1 report feedback
- Module 1 reports
  - Draft will be returned in ~ 1.5 weeks (M2D4)
  - Final revision due after spring break (M2D6)
  - Up to 1+1/3 letter grade improvement

# Mod 2 Goals, D1-4: background

## Design an siRNA



(Image from Wikimedia Commons, user Giac83)



Read and present  
about a specific  
topic in **RNAi**

Office ClipArt

## Learn **cell** culture (mammalian)

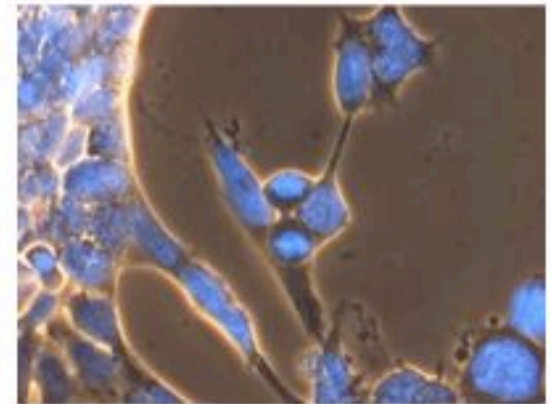


Image from [http://www.stemcellresources.org/library\\_images.html](http://www.stemcellresources.org/library_images.html)

# Mod 2 Goals, D5-8: experiments

siRNA against **luciferase**:

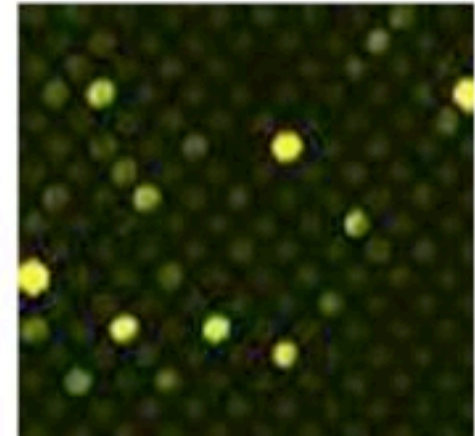
compare efficacy of designs  
use of a reporter plasmid  
basic statistics



Image from flickr user jon.nelson,  
license: <http://creativecommons.org/licenses/by-nc-sa/2.0/deed.en>

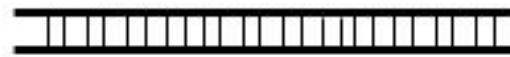
siRNAs against **mouse genes**:

direct, indirect, off-target effects  
analysis of large data sets

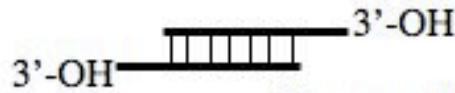


# Intro to siRNA

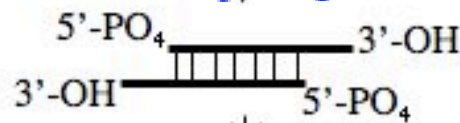
dsRNA



Dicer, ATP



Kinase, ATP



siRNA

binding and  
destruction of  
target mRNA

Design Goals

\* specificity

→ BLAST

\* efficacy

→ accessible  
(TD)

# Tissue Culture (TC) Environment

- What will “feel” physiological to a cell?

$T = 37^{\circ}\text{C}$

humidity

salts (cells could burst or shrink)

$\text{pH} = 7.2 - 7.4$



$\text{CO}_2 (5\%)$

ambient  $\text{O}_2$

\* sterility \*

# Tissue Culture (TC) Medium

- What do cells need to survive?

food: glucose and/or glutamine - energy  
or  
cell  
life  
(optional: Napyr)

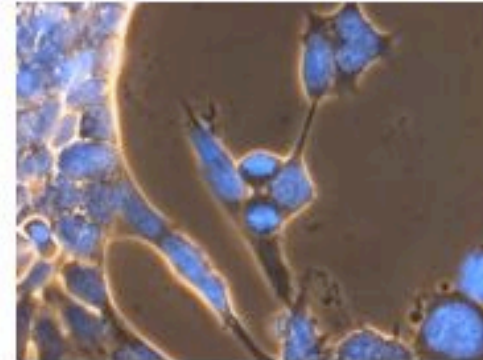
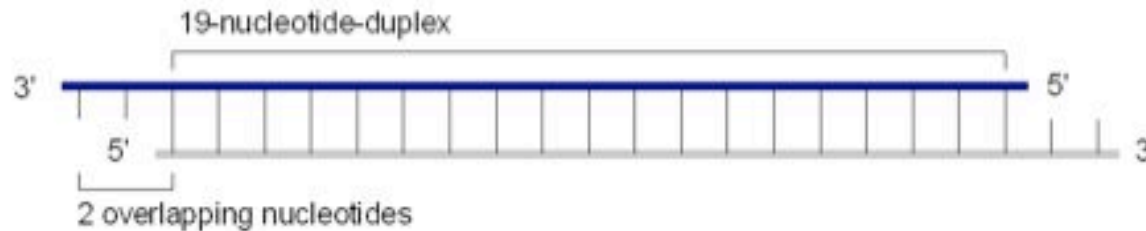
essential amino acids } building blocks,  
(optional: non-essential aa) } co-factors  
vitamins, minerals, lipids } for rxns.

serum → cytokines (e.g., growth factors)

non-food: antibiotics (Pen/Strep); phenol red - pH indicator

# Today in Lab: Workflow

Half start with siRNA design, half with cell culture



- For next time:
  - Read journal article for group discussion *45min.*
  - Start choosing article and day for solo presentation