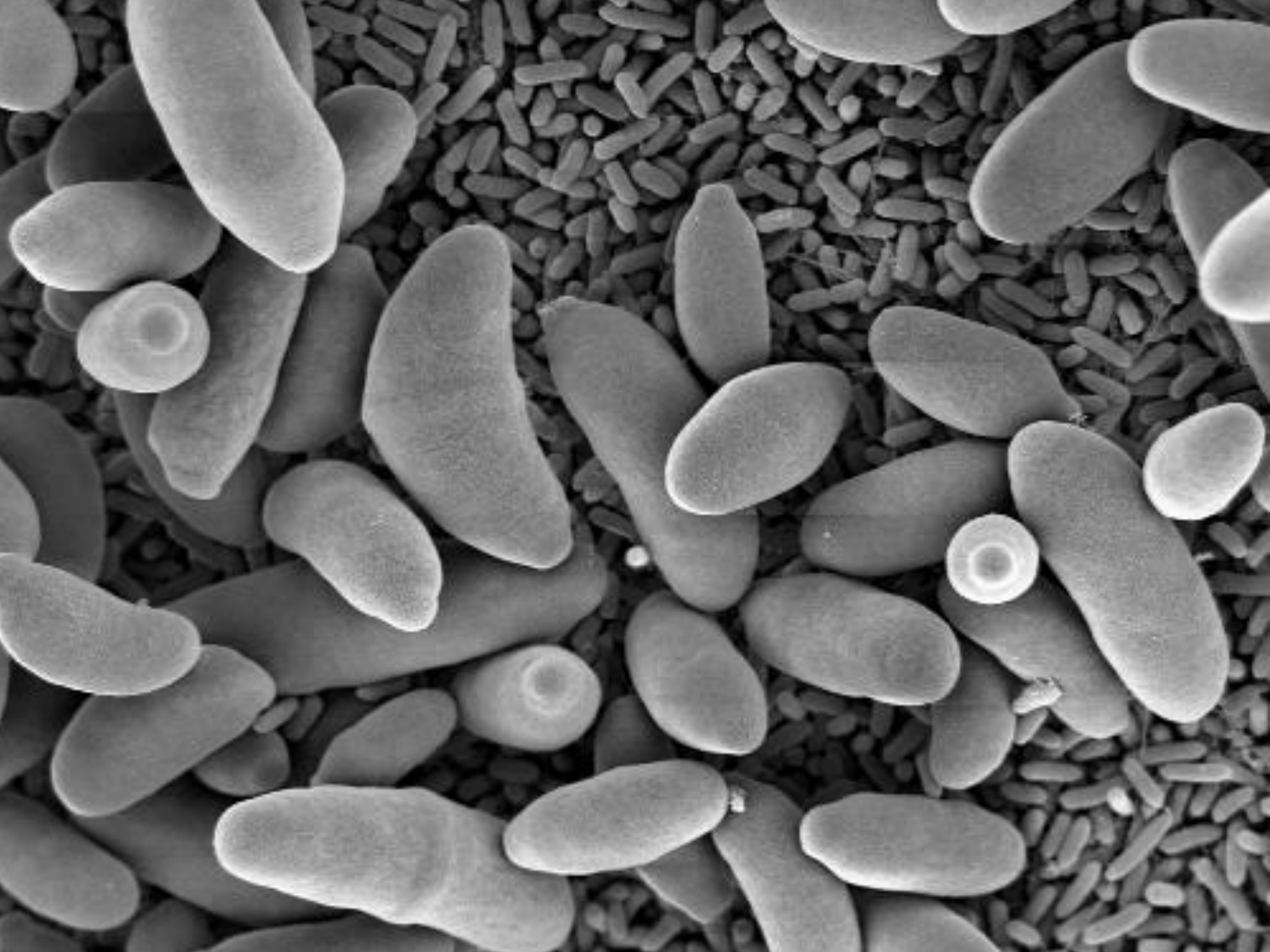
IPRO 302

Synthetic Biology

Engineering Novel Organisms



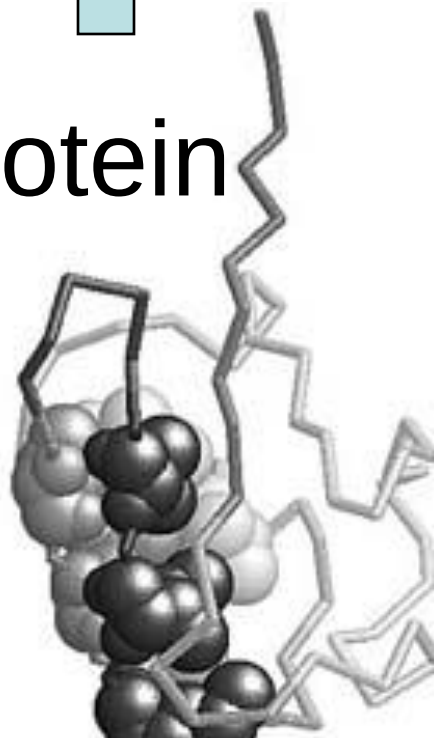
DNA

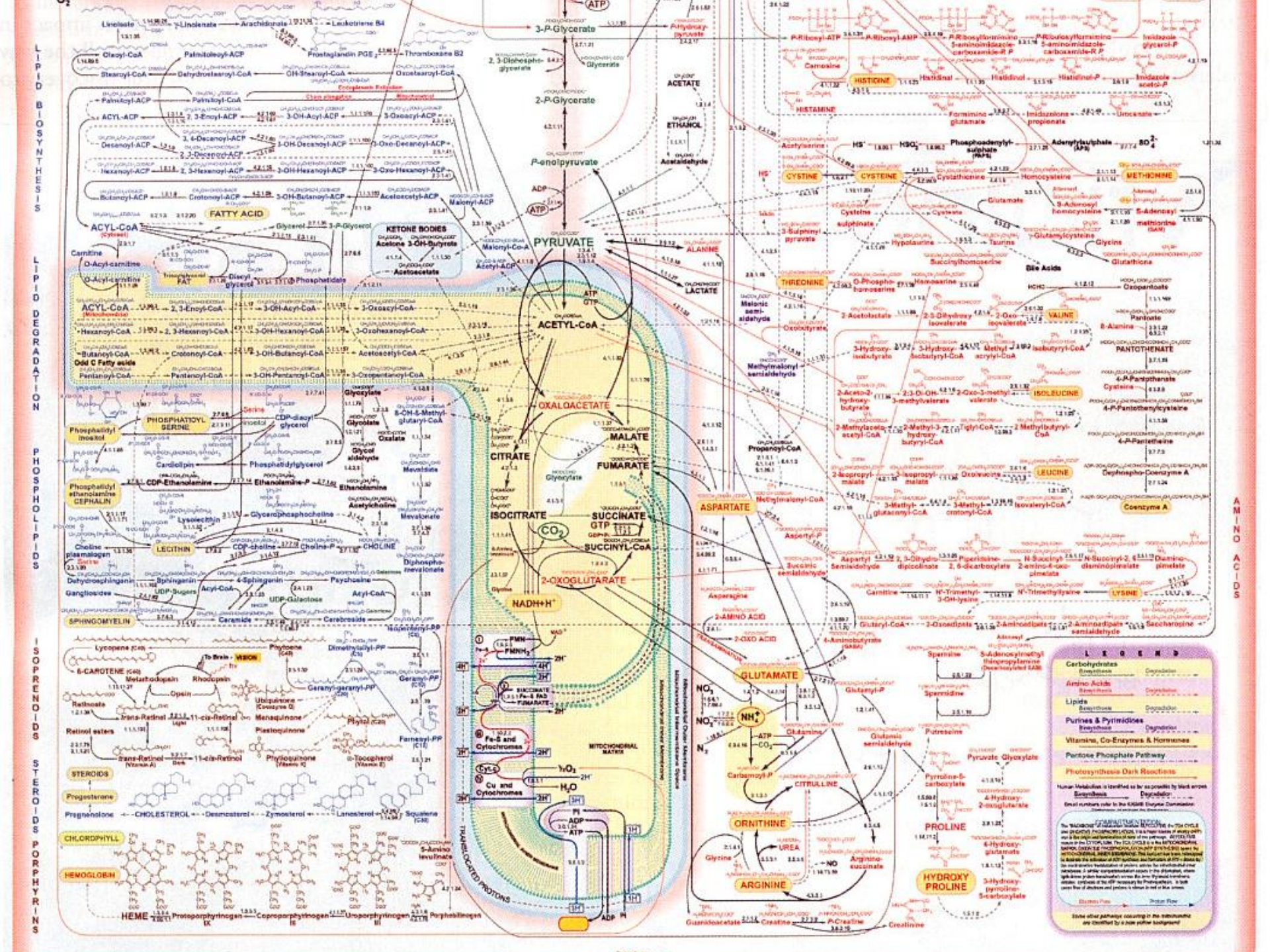
TCCCATGTGGGCAGCCGTTGGGACCTGCCAAGCACTGCGCCTAGT
TGAGCCAGTCCATGGGGAGGAGCCGTCCACCAGGCAGGCTATGCC
GTGCTGAATTCAGATGAGCTCCAGGAGTTGTACGAAGGCCTGAGG
AAATATGACTACGTGCTCACAGGTTATACGAGGGACAAGTCGTTC
K Y D Y V L T G Y T R D K S F
GACATTGTGCAGGAGCTGAAGCAGCAGAACCCCAGGCTGGTGTAC
D I V Q E L K Q Q N P R L V Y
TTGGGTGACAAGTGGGACGGCGAAGGCTCGATGTACGTCCCGGAG
L G D K W D G E G S M Y V P E
TACAAAGAAAAAGTGGTGCCGCTTGCAGACATTATCACGCCCAAC
Y K E K V V P L A D I I T P N
TTACTGAGTGGCCGGAAGATCCACAGCCAGGAGGAAGCCTTGCGG

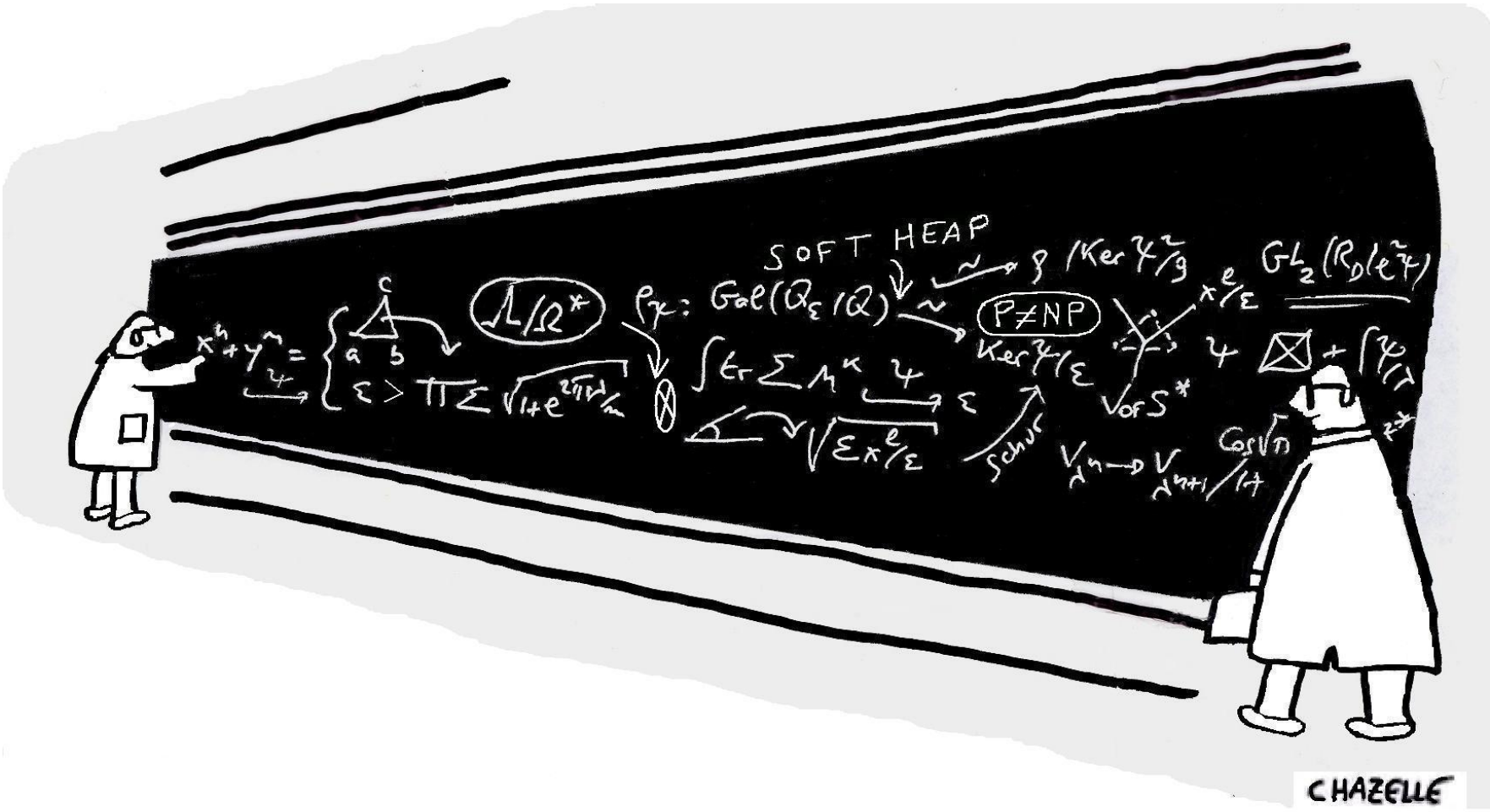
Code

Life

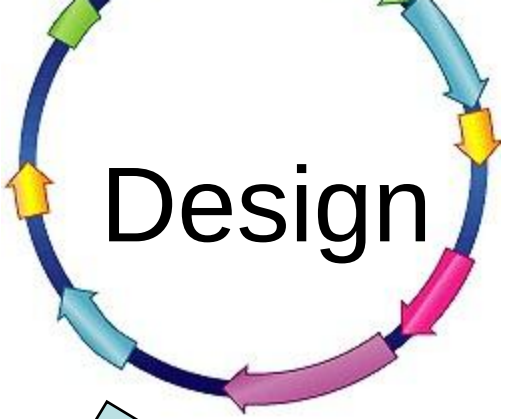
Protein




LIPID BIOSYNTHESIS
LIPID DEGRADATION
PHOSPHOLIPIDS
ISOPRENOIDS
STEROIDS
PORPHYRINS
AMINO ACIDS
FATTY ACID
ACYL-CoA
KETONE BODIES
PYRUVATE
ACETYL-CoA
OXALOACETATE
CITRATE
ISOCITRATE
2-OXOGLUTARATE
SUCCINYL-CoA
SUCCINATE
FUMARATE
MALATE
PHOSPHATIDYL SERINE
LECITHIN
CHOLESTEROL
GLUTAMATE
ASPARTATE
ALANINE
LACTATE
THREONINE
CYSTINE
CYSTEINE
METHIONINE
VALINE
ISOLEUCINE
LEUCINE
LYSINE
HISTIDINE
ARGININE
ORNITHINE
CITRULLINE
PROLINE
HYDROXY PROLINE
PANTOTHENATE
Coenzyme A
HEME
HEMOGLOBIN
CHLOROPHYLL
MITOCHONDRIAL MATRIX
TRANSLOCATED PROTONS
LEGEND

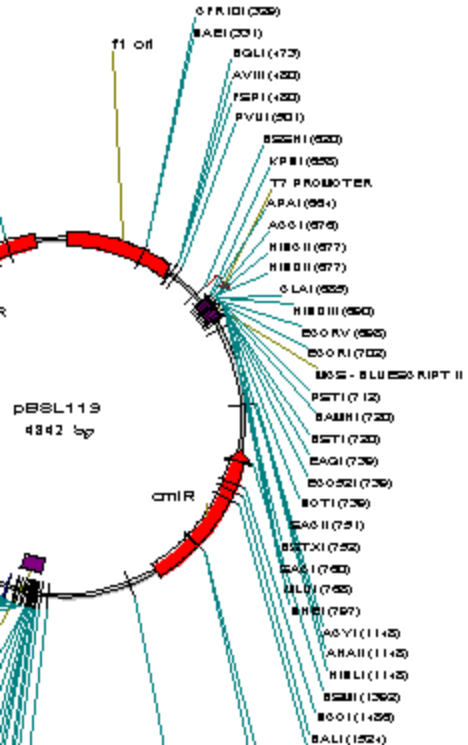


Too hard to understand!!

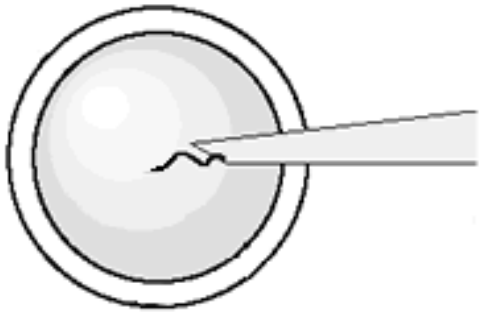


Build

Observe

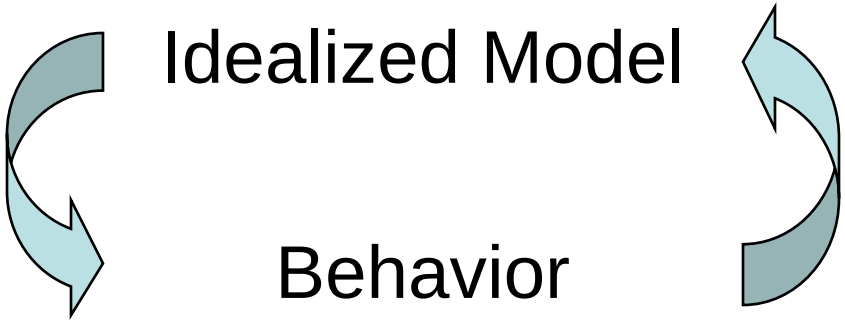


Implement



Modeling Overview

- Design to Specification
  - Create a Virtual Organism



Our target: a “rock-paper-scissors” oscillator in ZebraFish

Idealized Model

- 6 coupled differential equations

mRNA Equations:

$dm/dt = -\beta_m m + \alpha_1 p^n/(k_d+p^n) +\alpha_0$

$dm/dt = -\beta_m m + \alpha_1 p^n/(k_d+p^n) +\alpha_0$

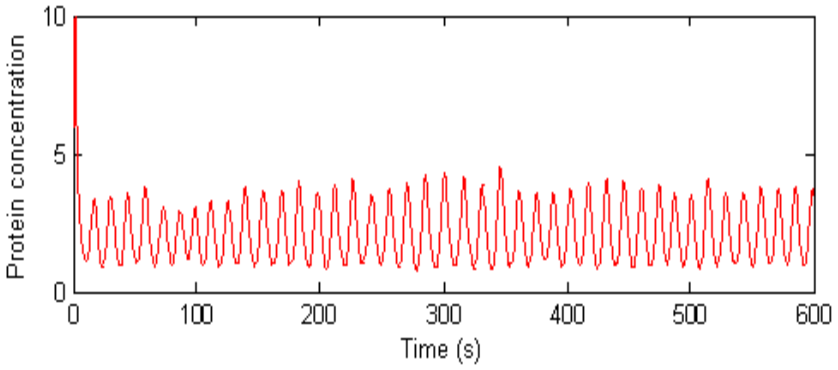
$dm/dt = -\beta_m m + \alpha_1 p^n/(k_d+p^n) +\alpha_0$

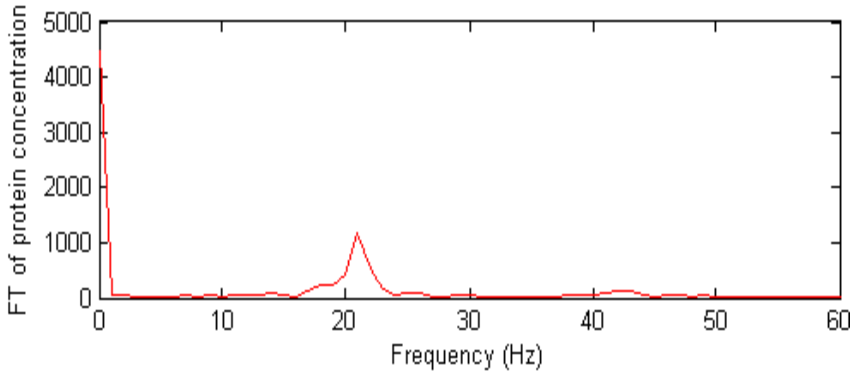
Protein Equations:

$dp/dt= t_s*m - bp*m$

$dp/dt= t_s*m - bp*m$

$dp/dt= t_s*m - bp*m$





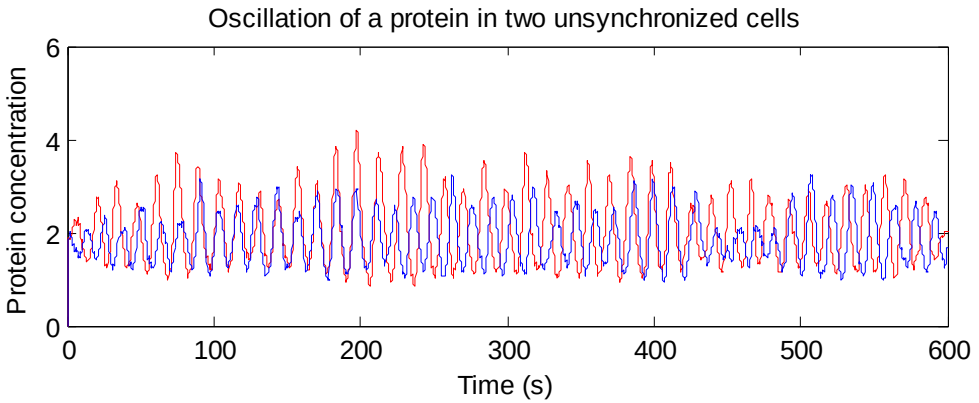
More Realistic

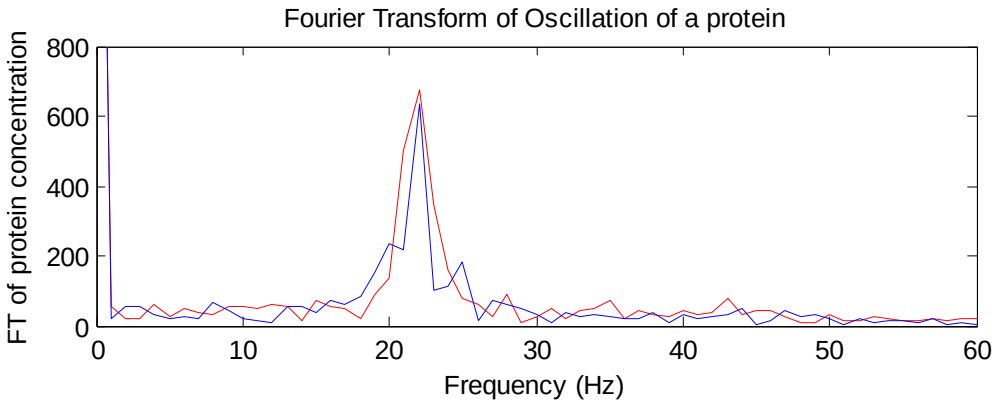
- Stochasticity

$$\lambda_m = -\beta_m m + \alpha_1 p^n/(k_d+p^n) + \alpha_0$$

$$P_{\lambda m}(n) = \lambda^n e^{-\lambda}/n!$$

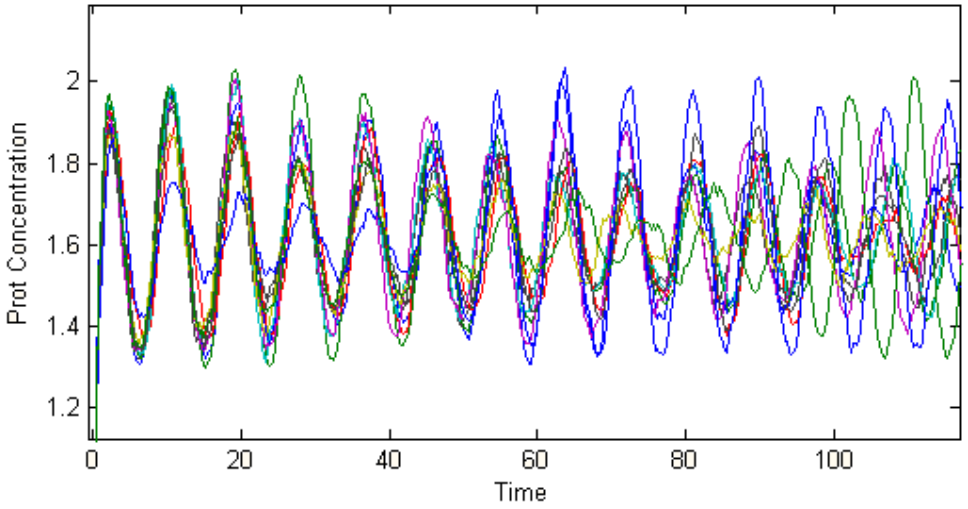
$$\Delta m/\Delta t = \text{rand}(P_{\lambda m}(n))$$





More Realistic II

- Multi-cellular Organism
  - Example: human or zebrafish
  - Making the whole organism act as one



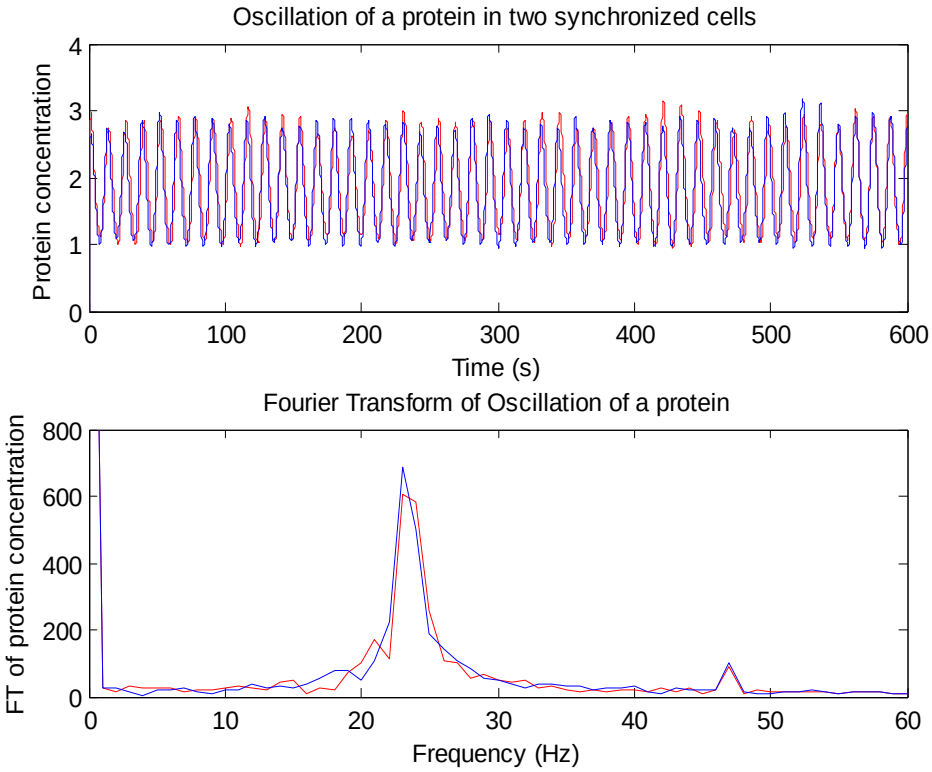
Inducing Synchronization

- Inducers are modeled

$$\frac{dS_{int}}{dt} = -k_{s0} S_{int} + k_{s1} p_{jk} - \eta (S_{int} - S_{ext})$$

$$\frac{dS_{ext}}{dt} = -k_{se} S_{ext} + \eta \sum_{n=1}^{N} (S_n - S_{ext})$$

- Synchronization:

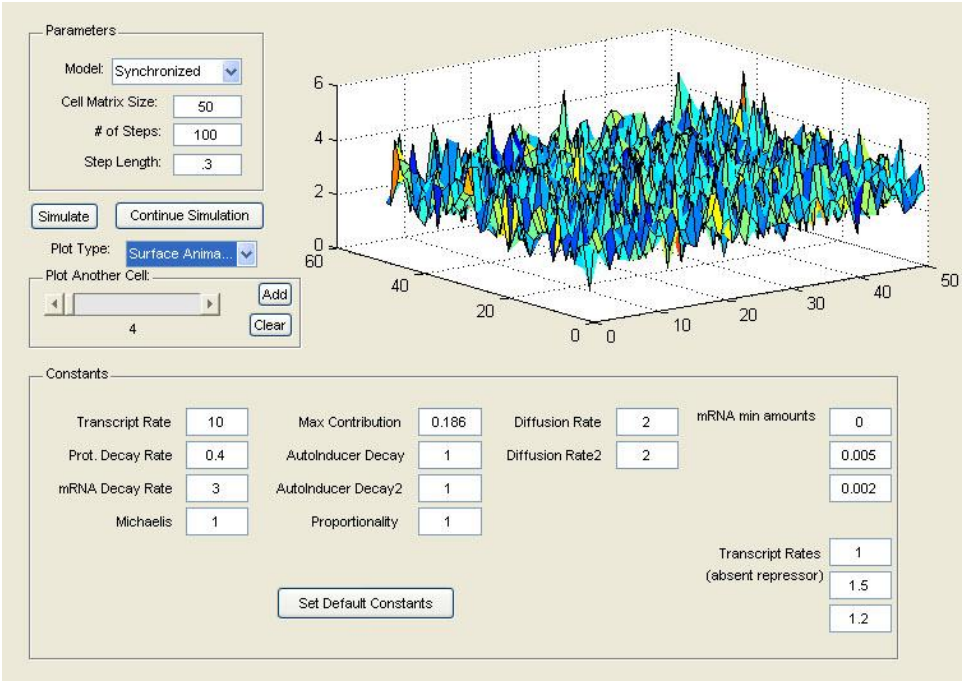


Past Work

- MATLAB
  - Easy to use, High-level
  - Slow
  - A small organism was modeled and synchronized
- C++
  - More difficult, Low-level
  - Much faster
  - Large Organisms can now be simulated

Our Progress

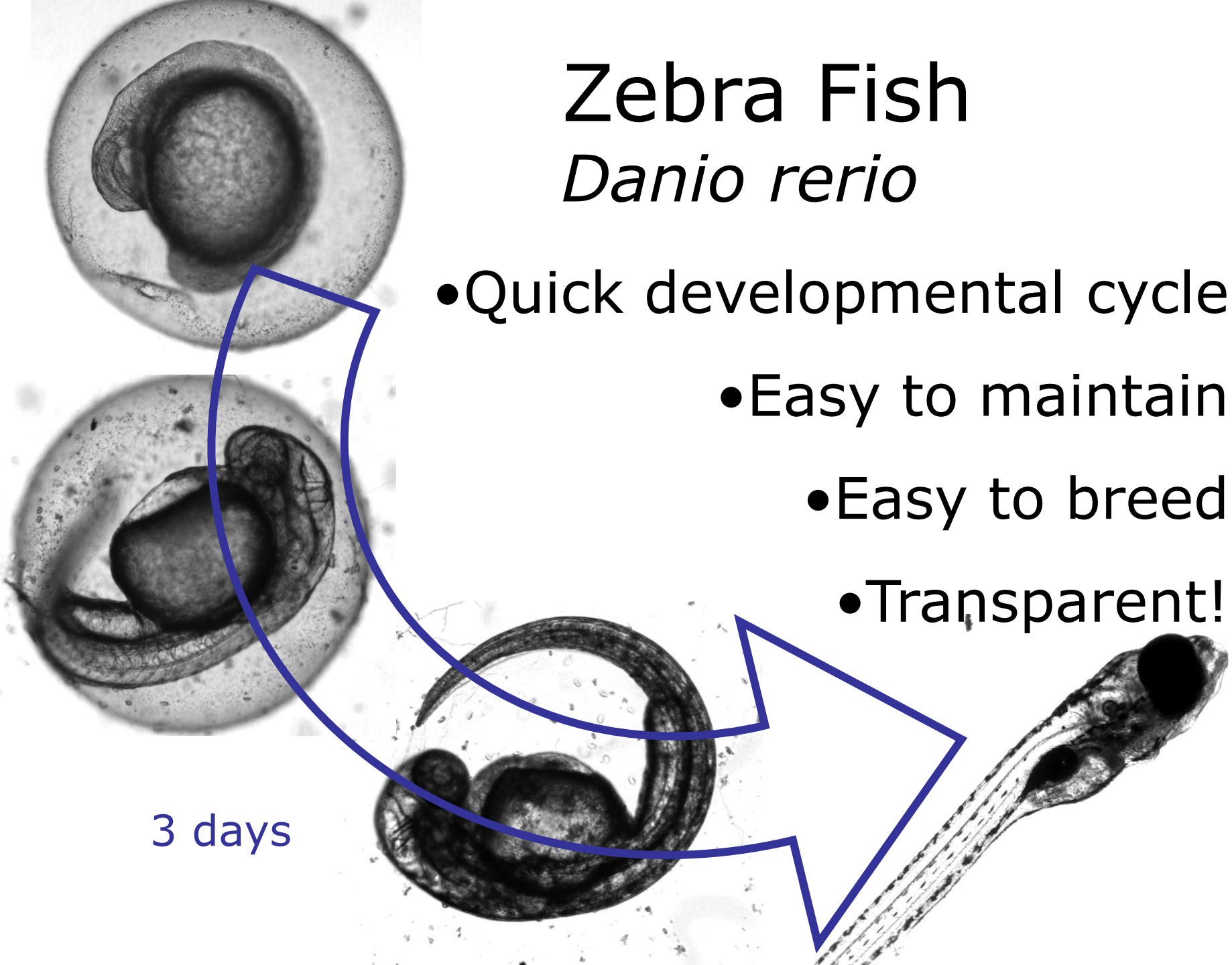
- Hybrid MATLAB/C++ program
  - C++ for heavy calculations
  - MATLAB for data analysis
- User Interface
  - Quick Operation
  - Visual Analysis



Zebra Fish

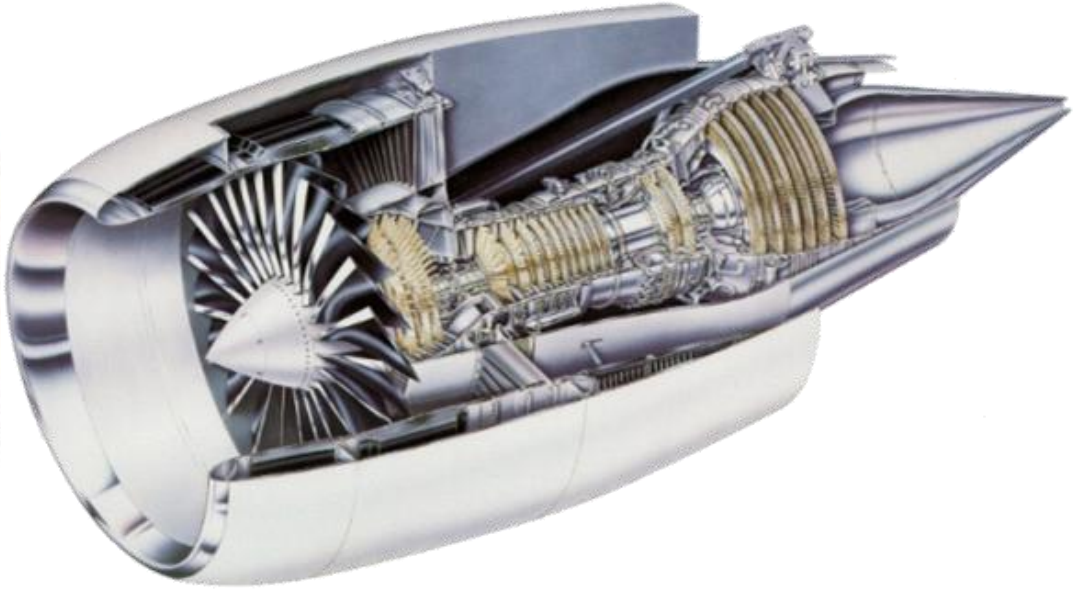
Danio rerio

- Quick developmental cycle
- Easy to maintain
- Easy to breed
- Transparent!



Advancement of the Genetic Circuit

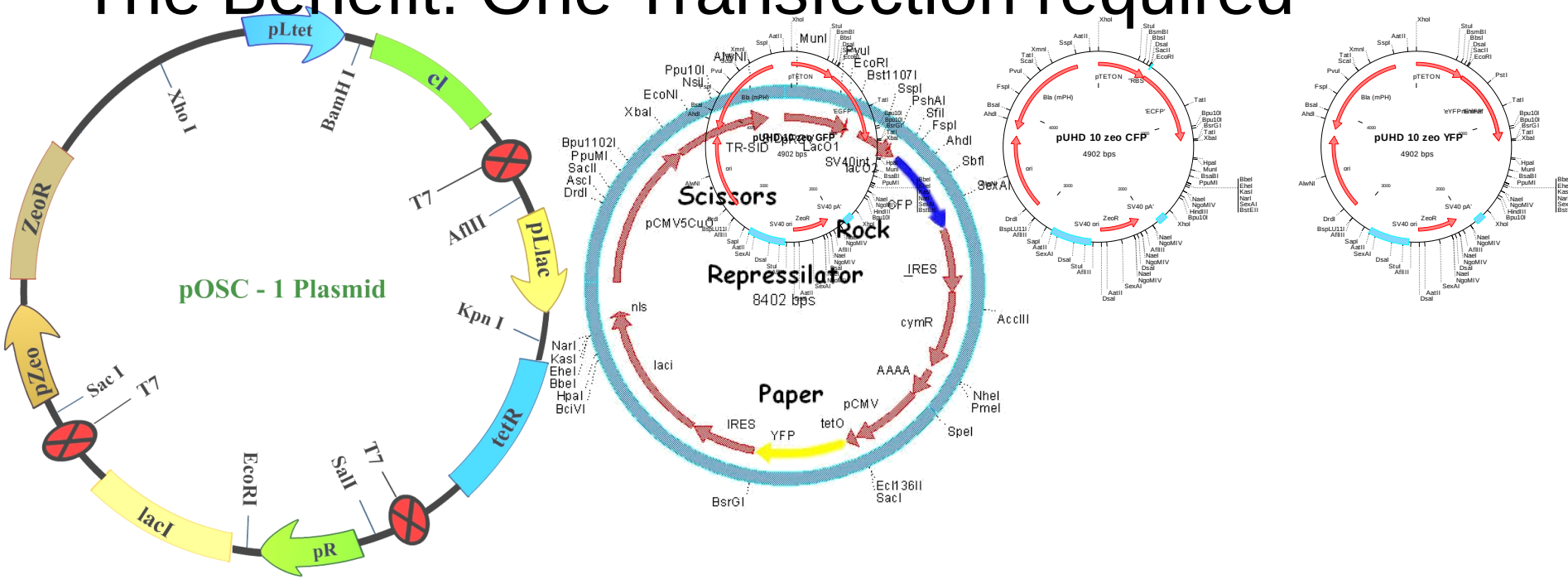
- Previous semesters created an Oscillatory circuit, which functioned in Bacteria
- Our goal is to remodel this circuit allowing it to function in higher order organisms: Eukaryotic Cells



Redesign of The Circuit

A new approach

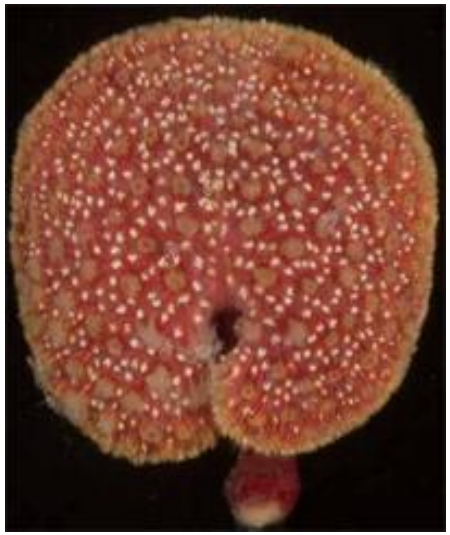
- Last Semester's Plasmid Oscillator
- Repressilator
- The Problem: Multiple transfection.
- The Benefit: One Transfection required



Assembling the Genetic Circuit

- Take genes found in different organisms and attach them together to create a completely unique DNA molecule.

Aequorea victoria



Renilla reniformis

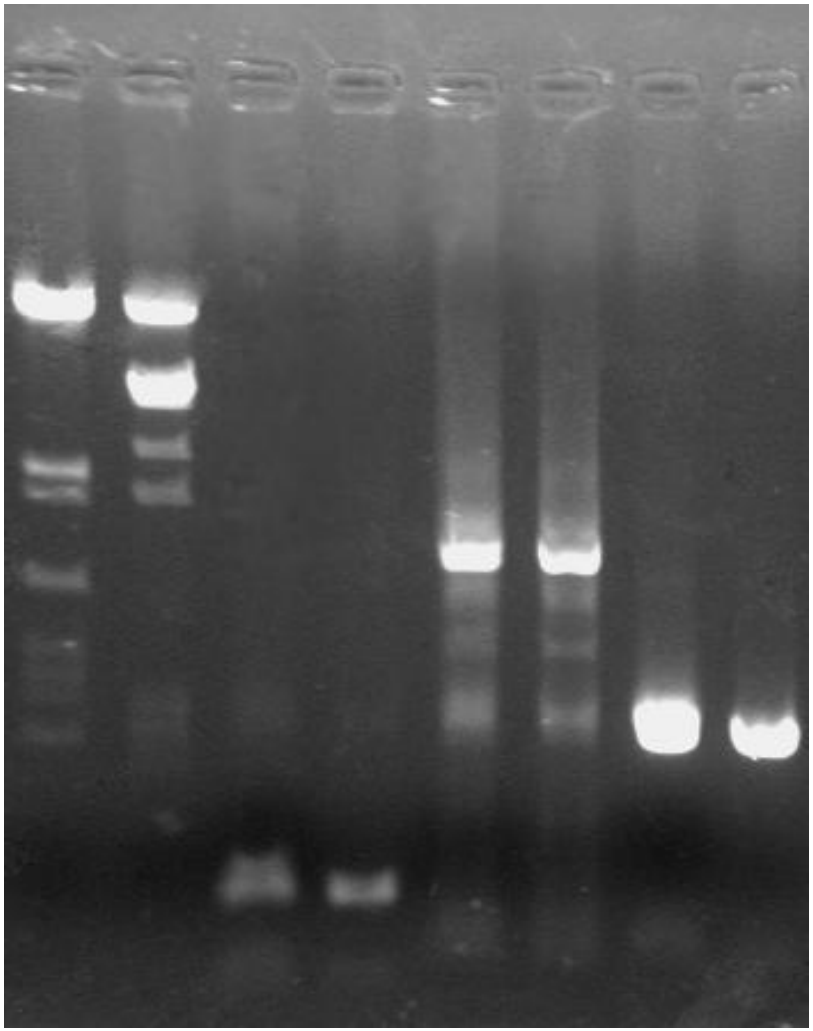
Discosoma

Component Construction

Fusion Polymerase Chain Reaction (PCR)

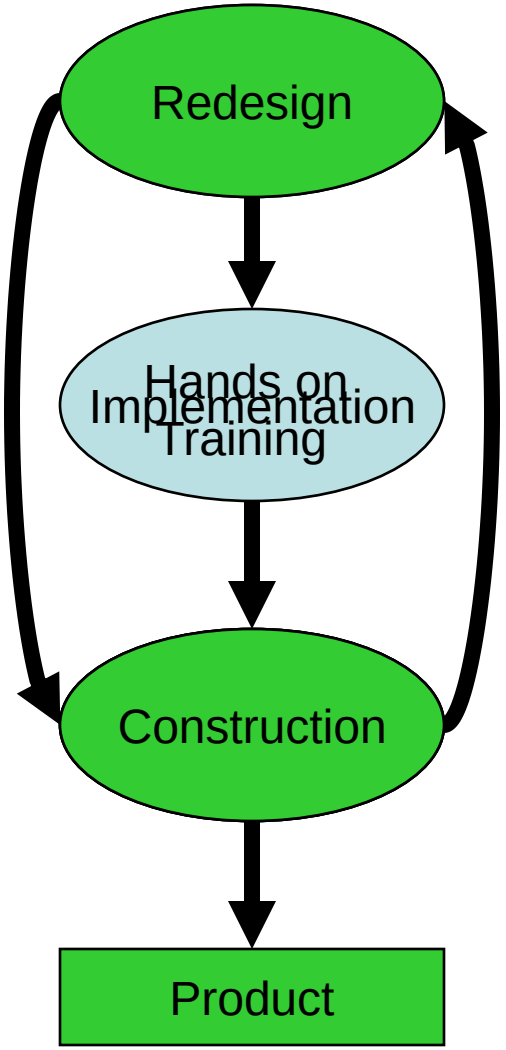
- Electrophoresis gels
- Primers: Short sequence of DNA that attach onto opposite ends of the gene.
- Bands show Base Pair length
- DNA polymerase: Enzyme that binds to the primer and builds complementary DNA strand.
- Excision of correct bands

- Fusion PCR
  - Special primers link strands.
  - DNA polymerase assembles new molecule composed of the two fragments



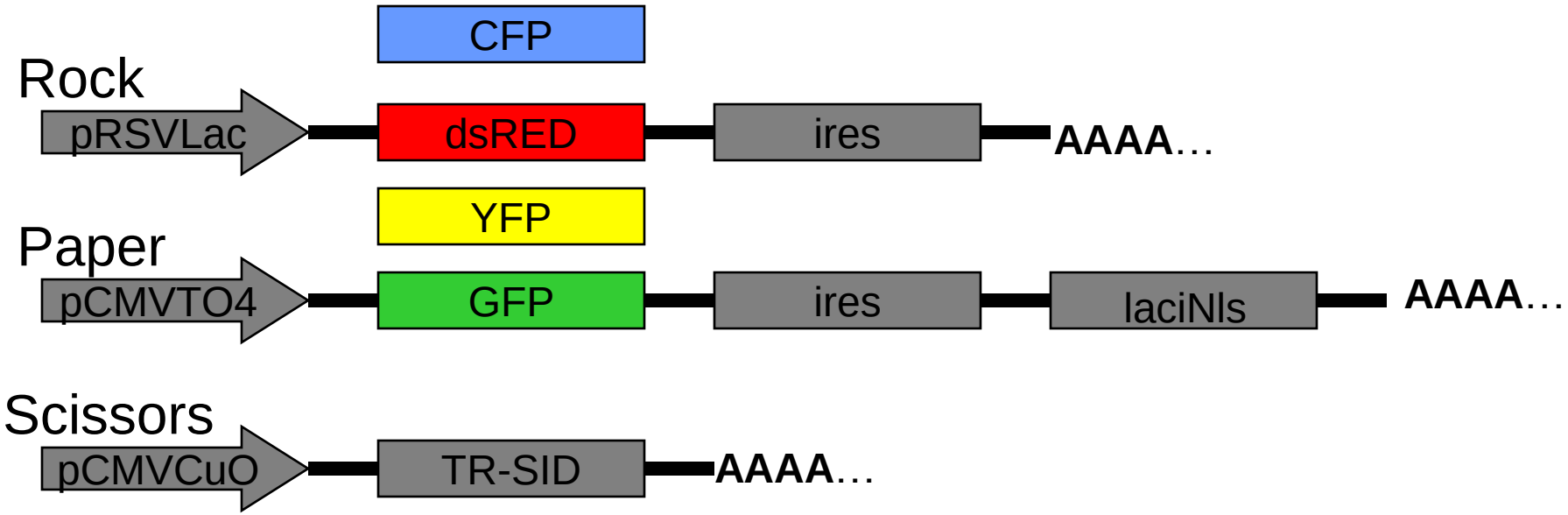
Obstacles We Encountered

- Training new members in laboratory procedure.
- The RFP gene sequence we ordered had modifications.



Redesign

- Redesign Fluorescent Proteins
- Redesign Primers
- Redesigned Protein (dsRED) failed
- Replaced dsred with Cyan Fluorescent Protein (CFP)
- Replaced Green with Yellow Fluorescent Protein (YFP)



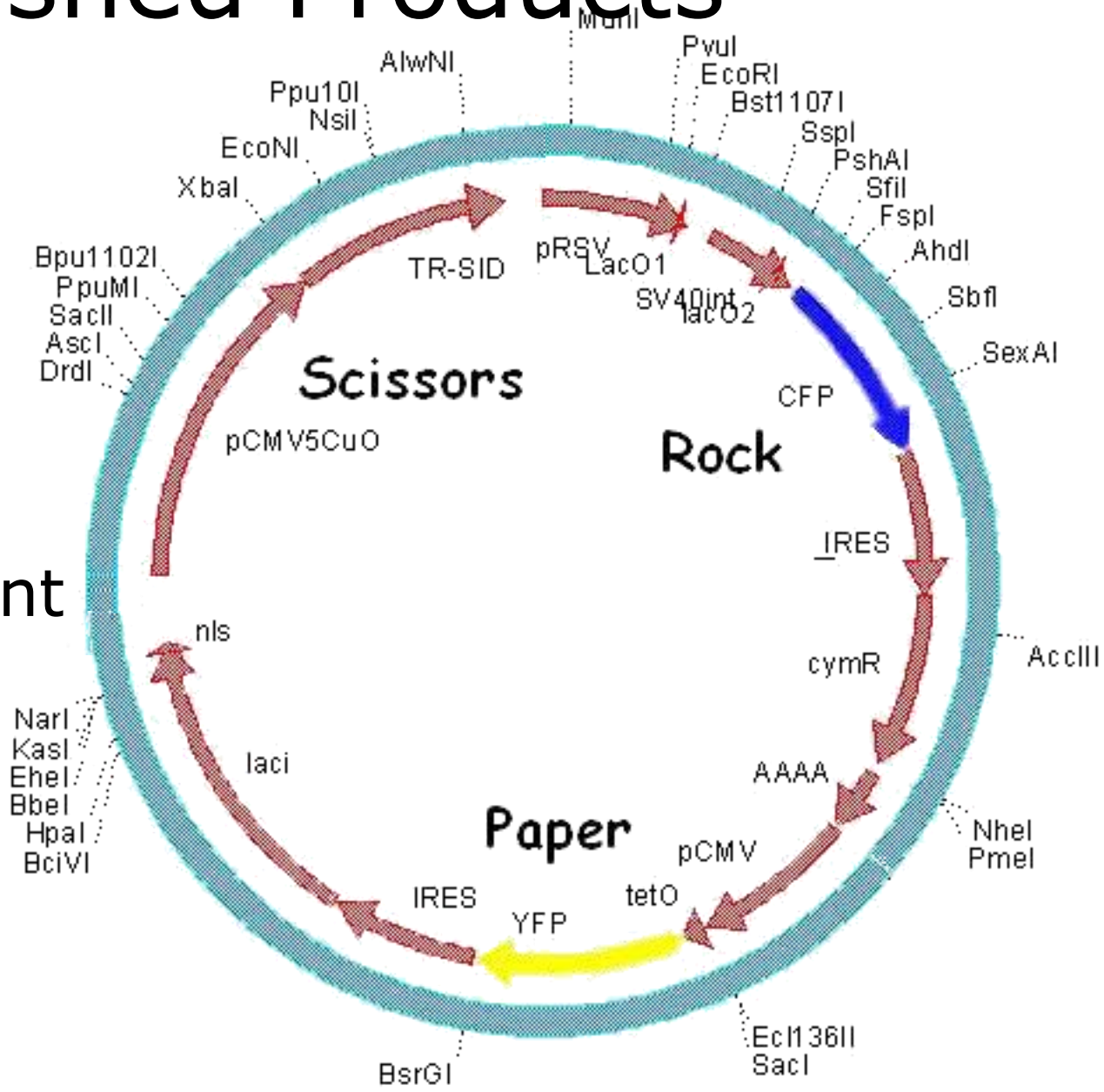
Finished Products

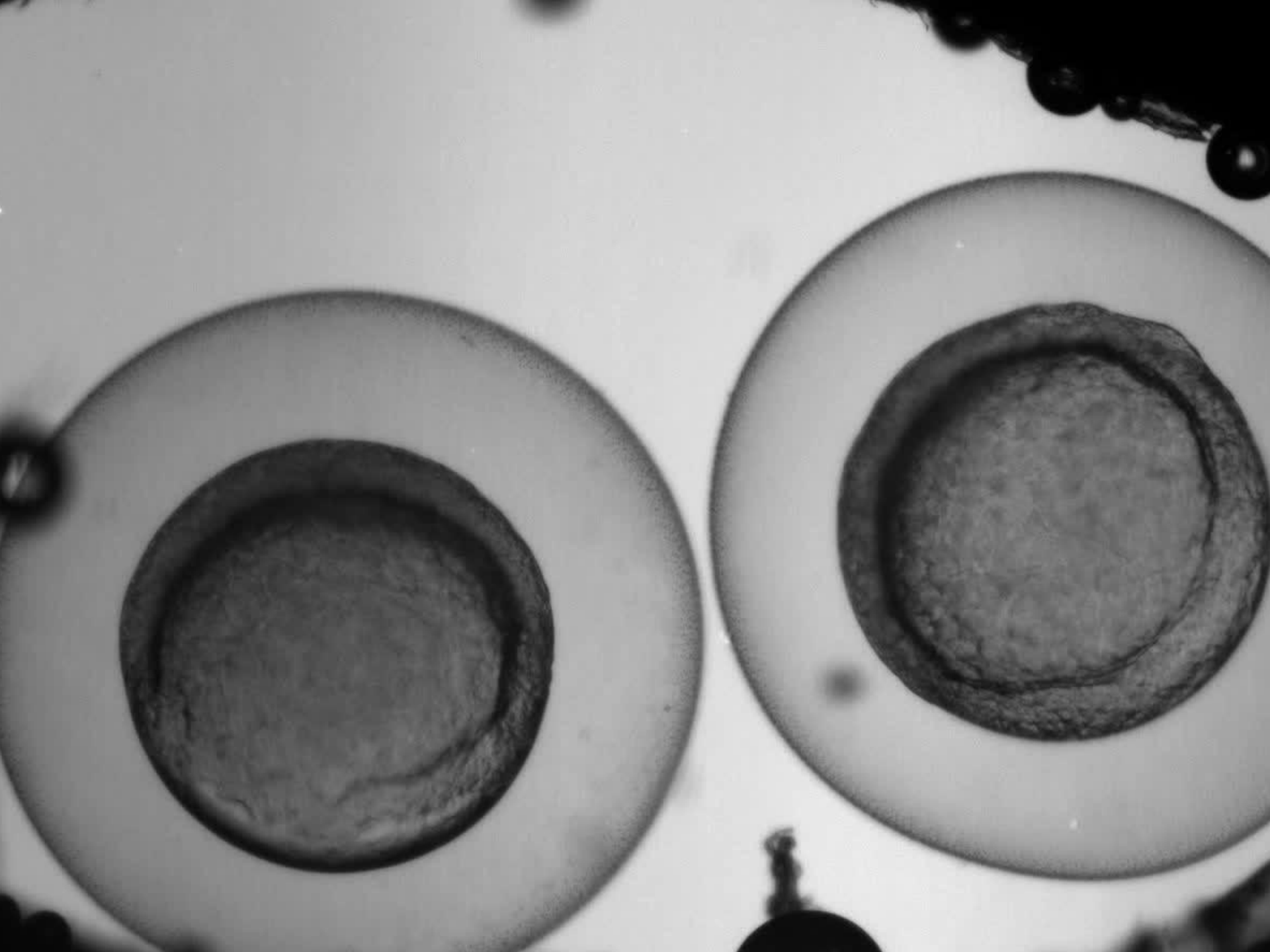
Paper Segment

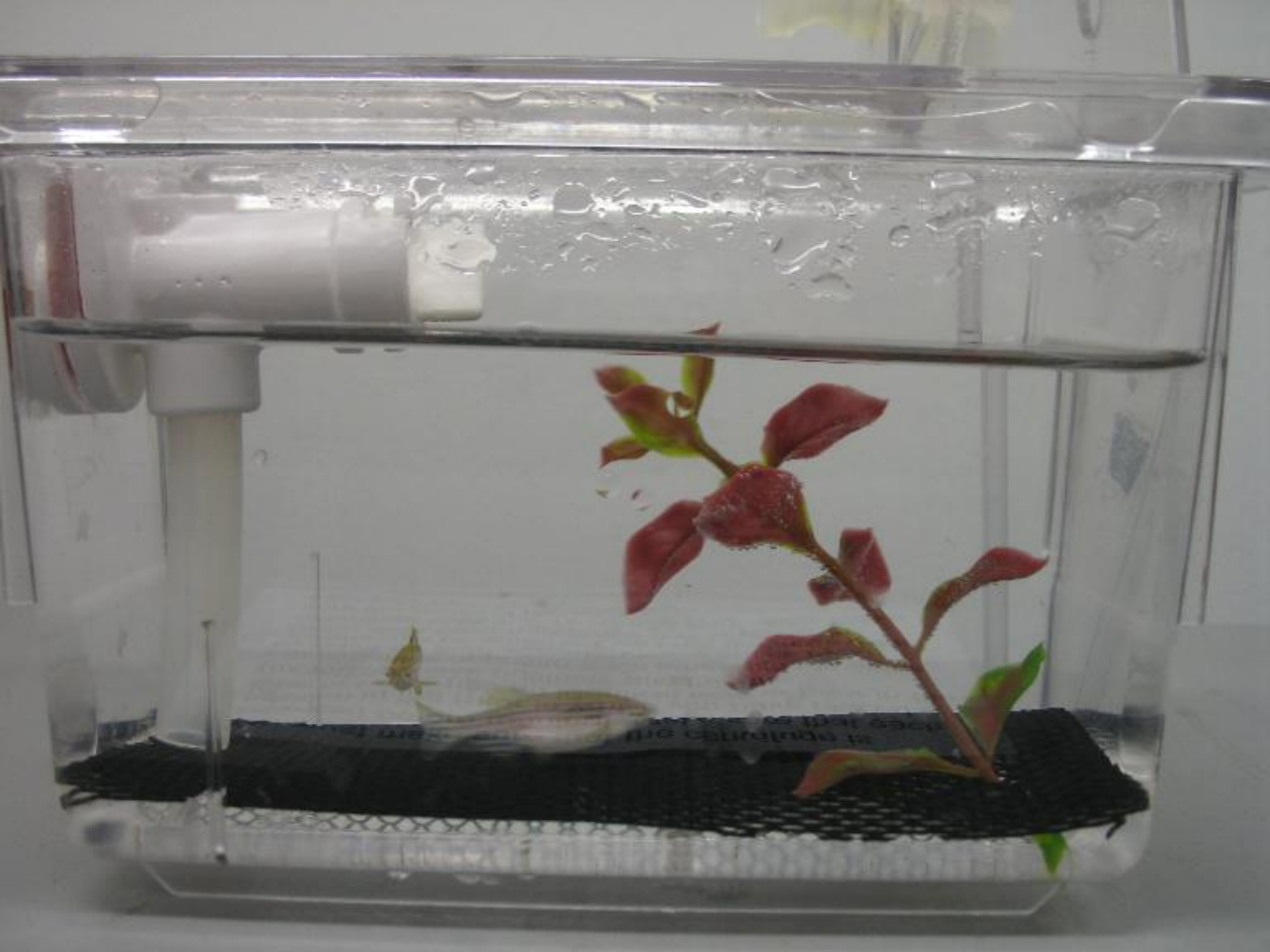
- Complete
- Isolated
- Awaiting confirmation

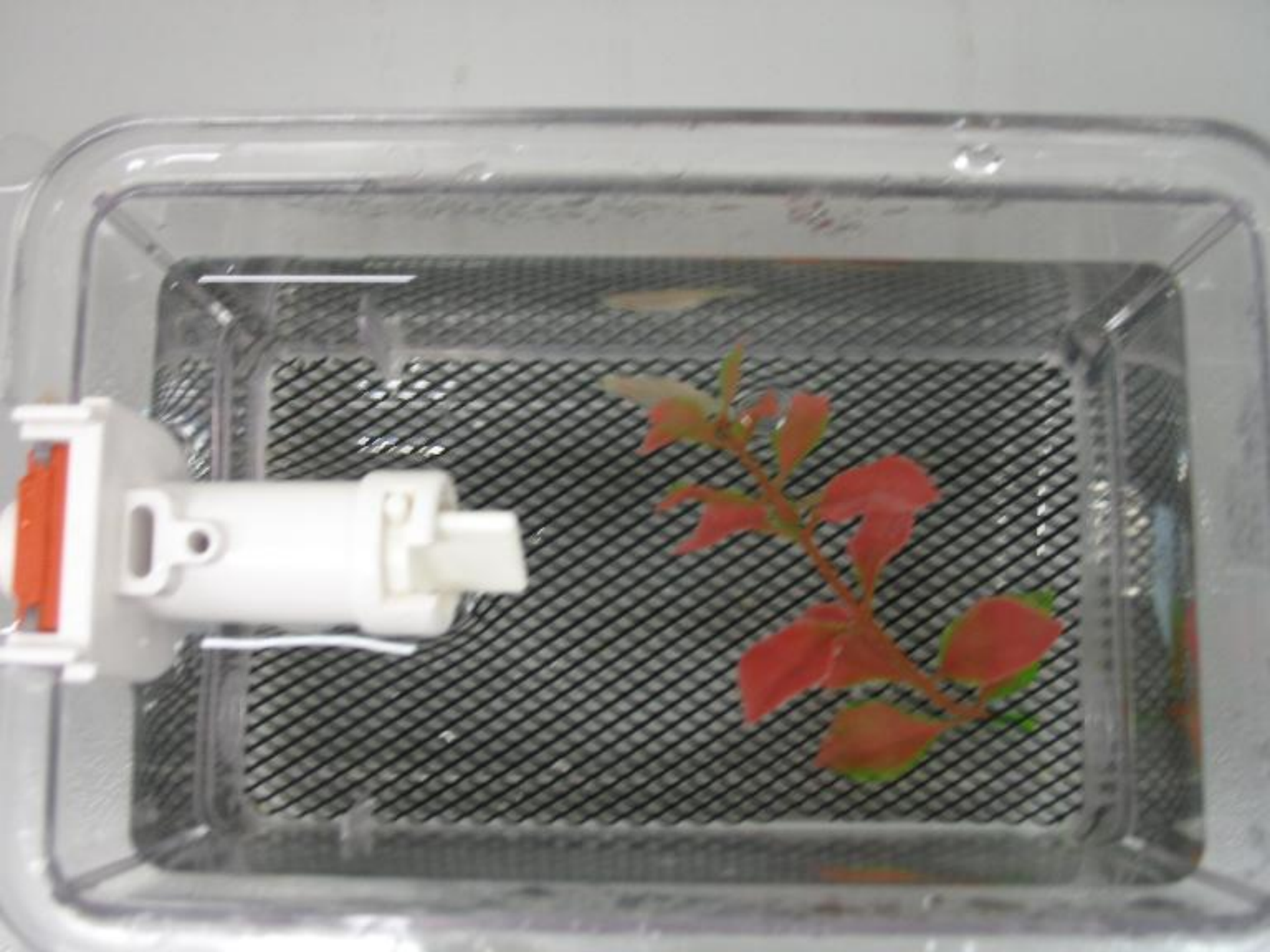
Scissors Segment

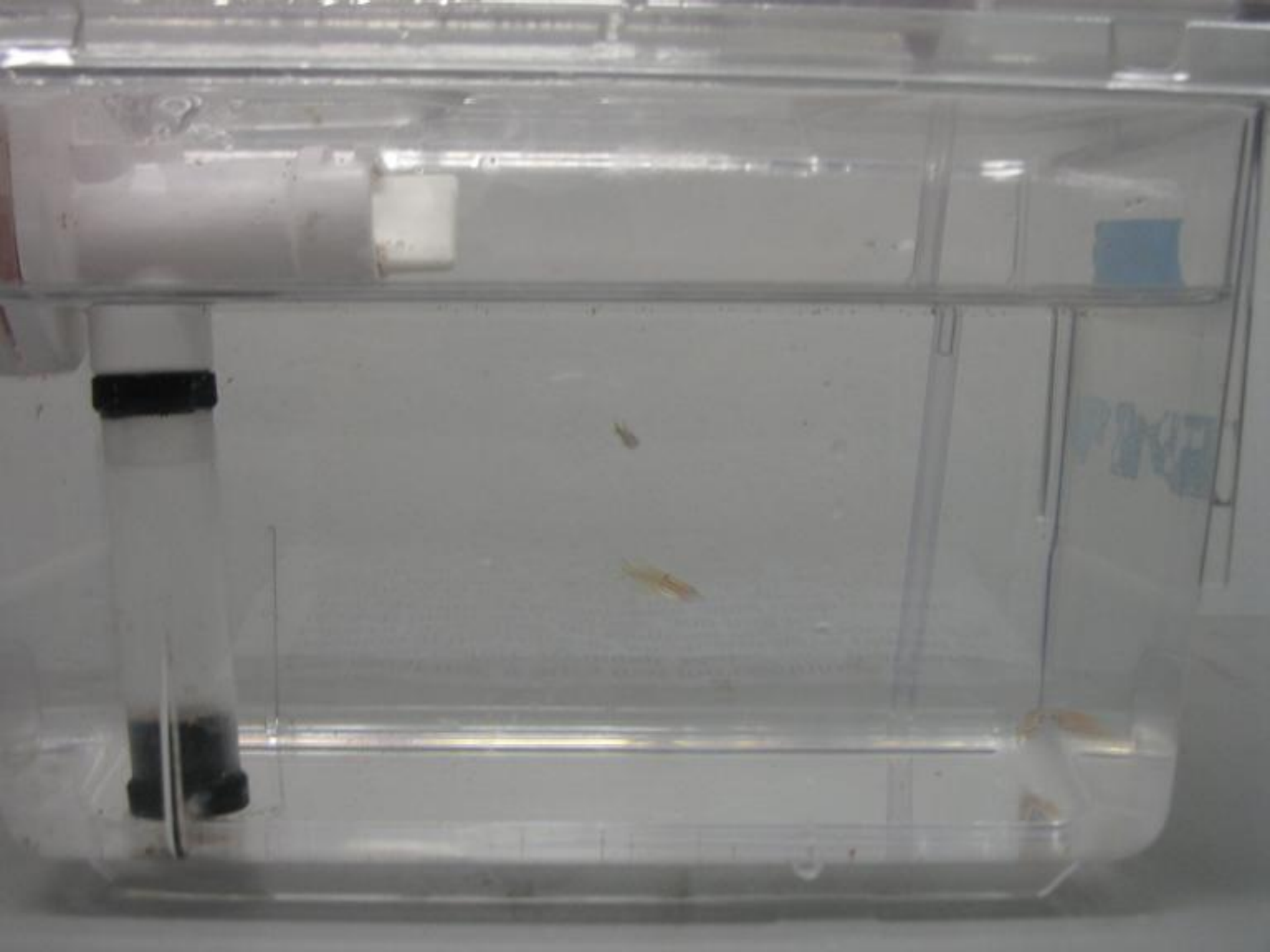
- Complete
- Isolated
- Ready for Insertion





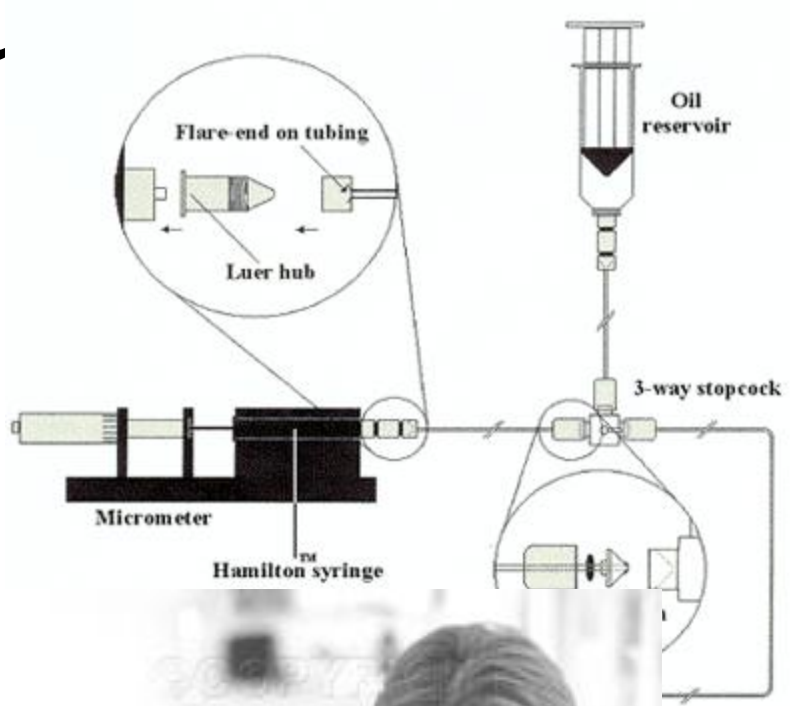


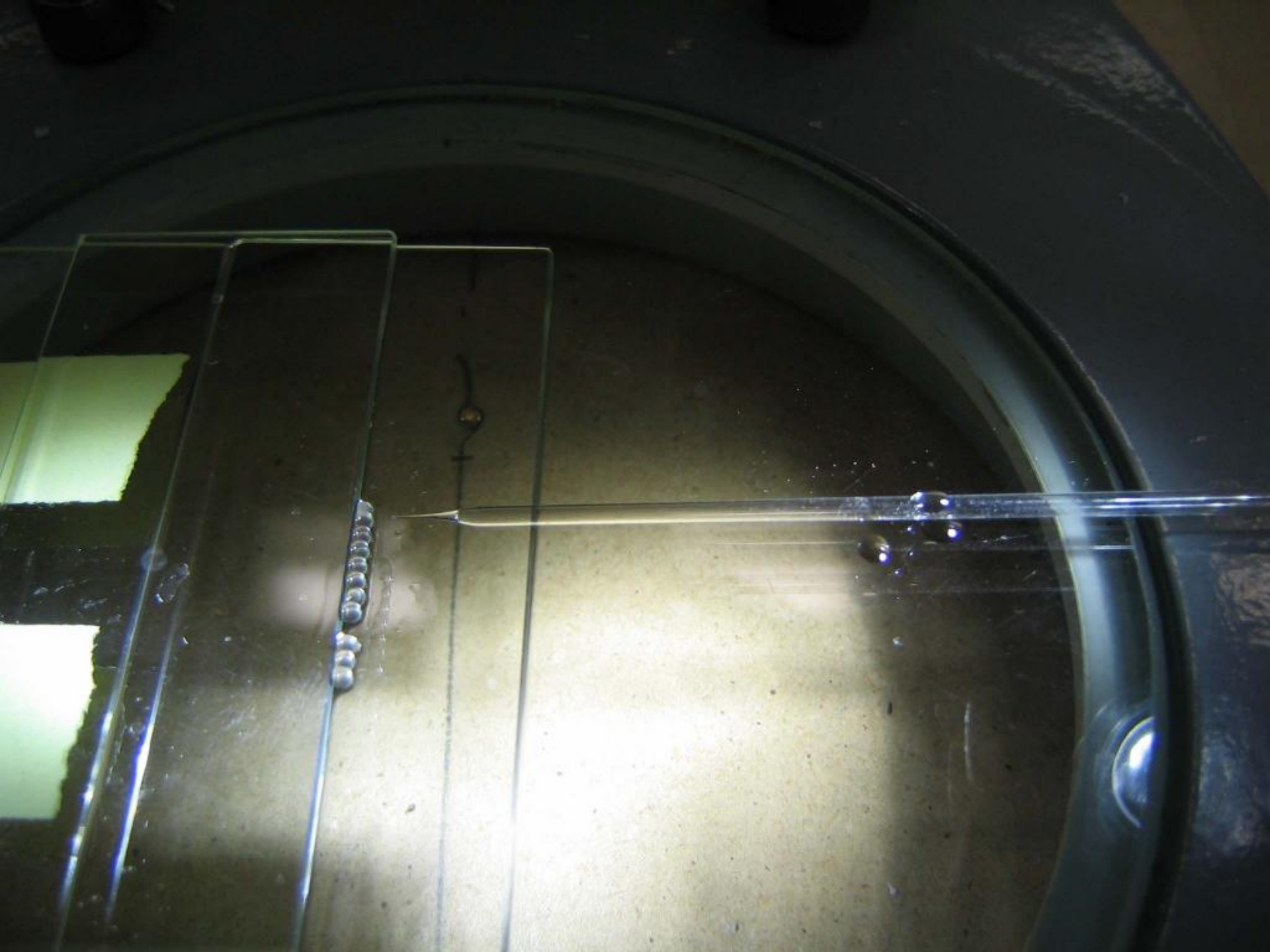


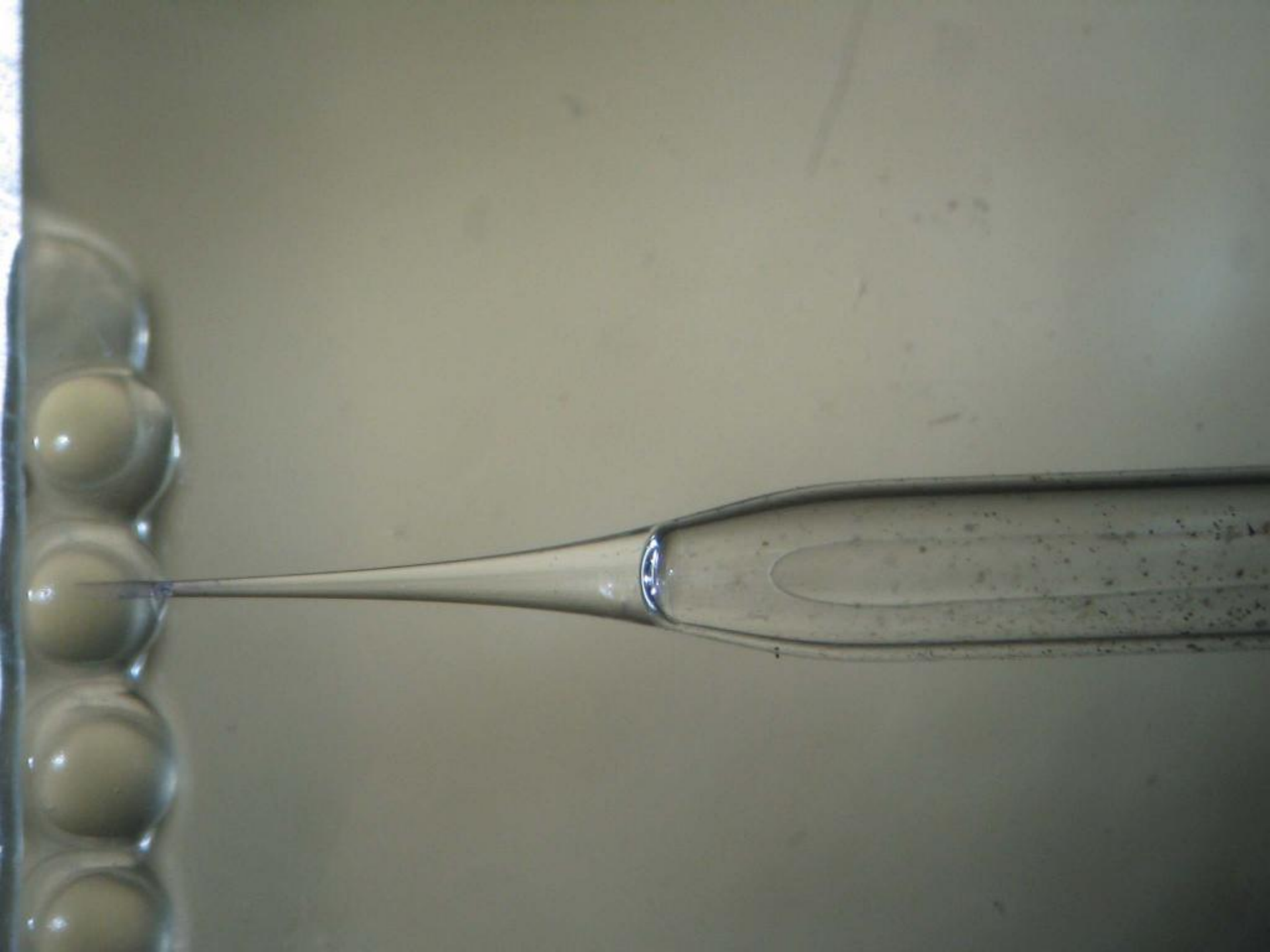


How do we get our DNA circuit in?

Micro Injection

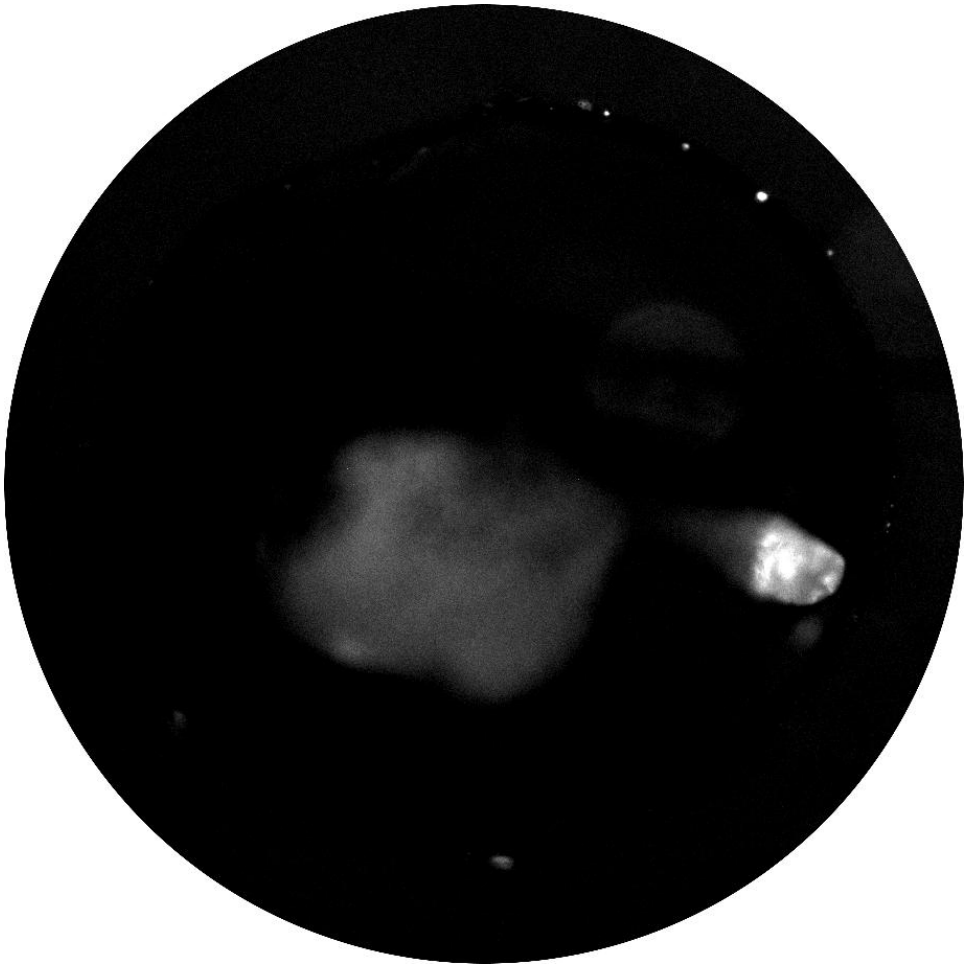






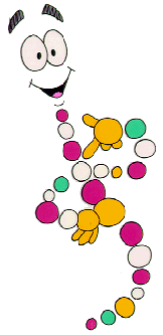
LEAD MCMP/MJPEG Decoder Eval

Brightfield image



Fluorescence image

Futures and Ethics



George is from the future

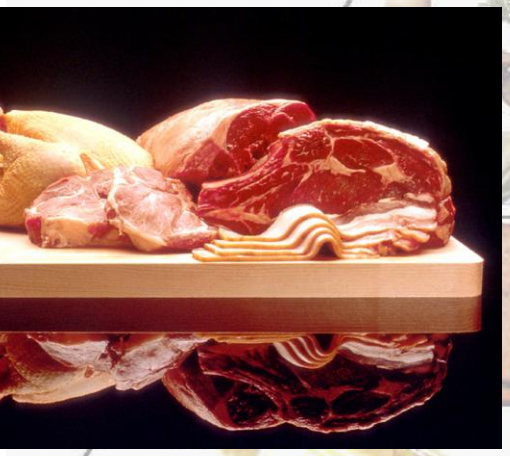
Bacteria could remove oil and dirt from skin.

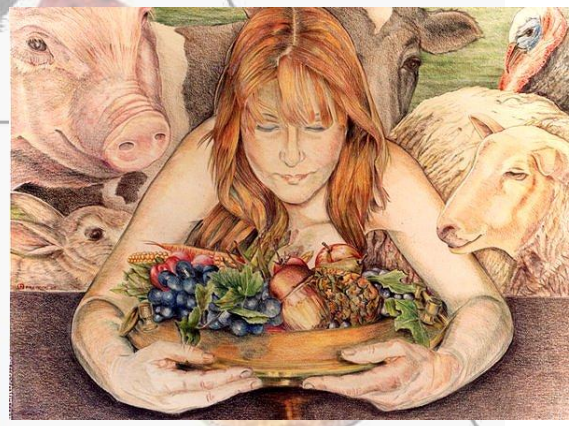
Many new organic materials could be produced on a large scale

+

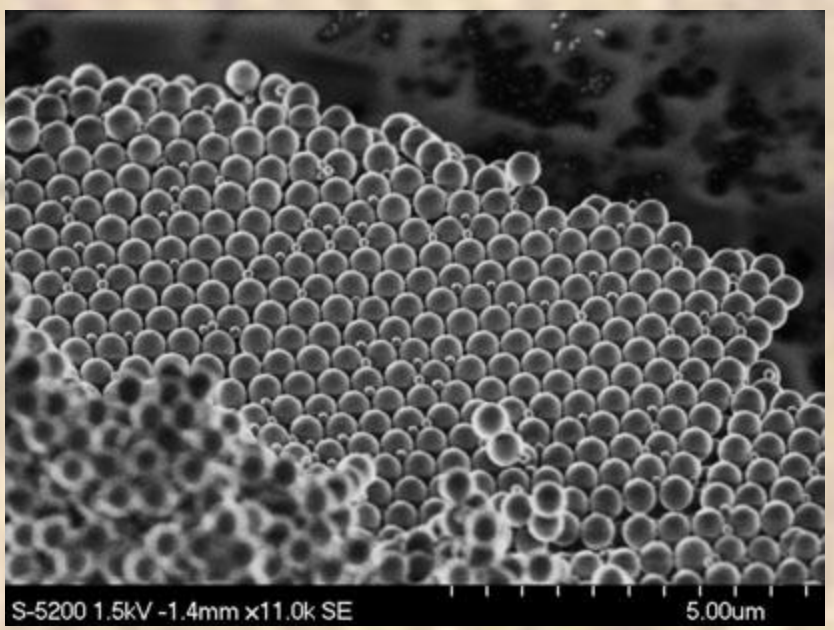
=

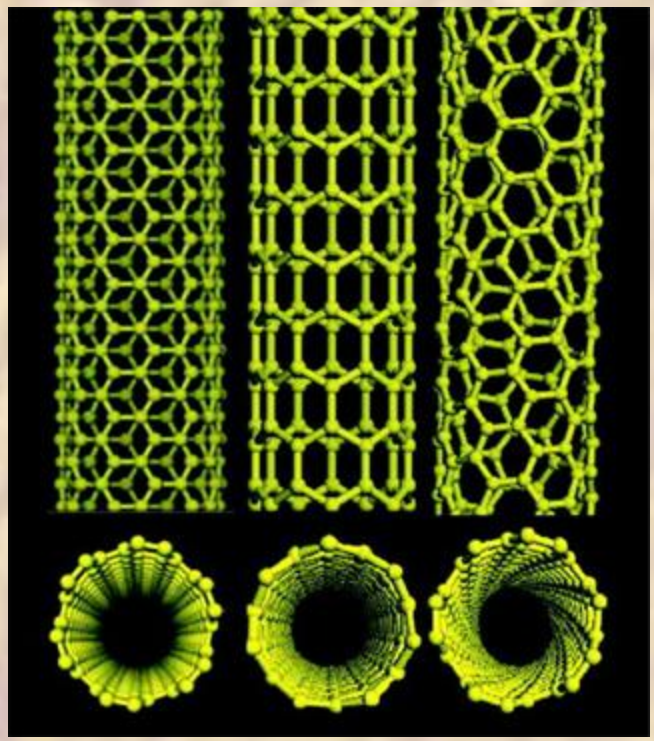
People could modify themselves around their diet, rather than their diet around their nutrient needs

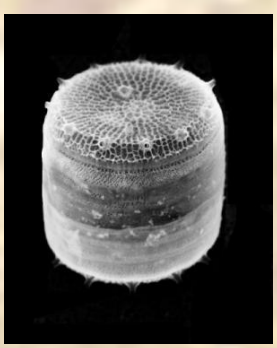




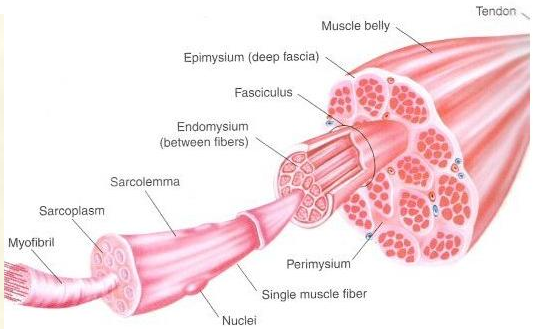
Microorganisms could be used to assemble nano-materials

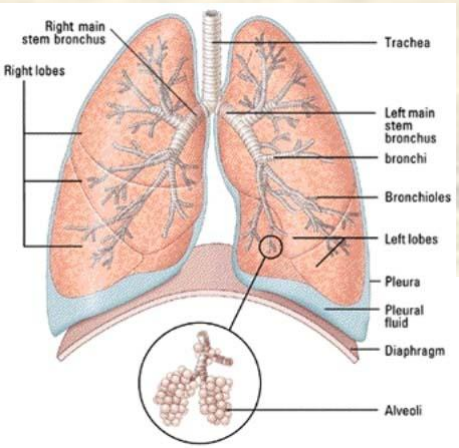






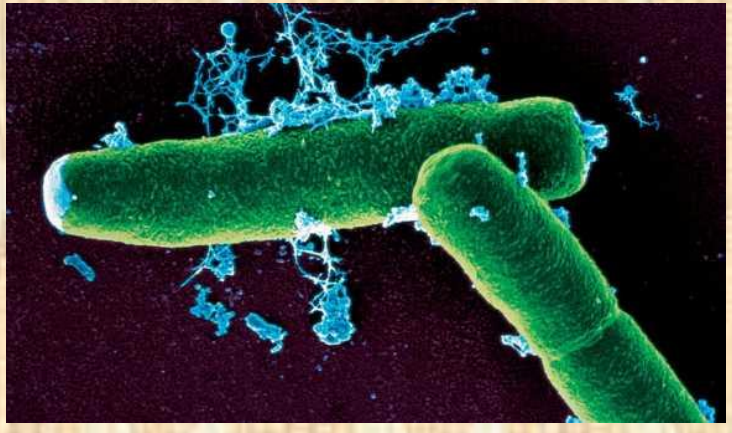
Profound changes could be made to human physiology





Potential Dangers

- Bioterrorism
- Genetic Pollution



Should genes be manipulated?

Teosinte

Gamagrass

Business and Research Ethics

- Who should how regulations should work?
  - Scientists?
  - Businesses?
  - Government?
- What regulations need to be made, or made better?

The Public needs to know

- What genetic engineering and synthetic biology are
- What they aren’t
- What the benefits and risks are

What could humans become with synthetic biology?

?