IPRO 302

Synthetic Biology
Engineering Novel Organisms

Our Motivation

- How does life “work”?

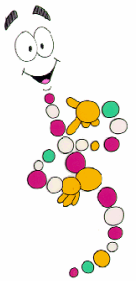
Observe

- DNA provides life’s blueprints

Understand

- Can we make our own blueprints?

Control

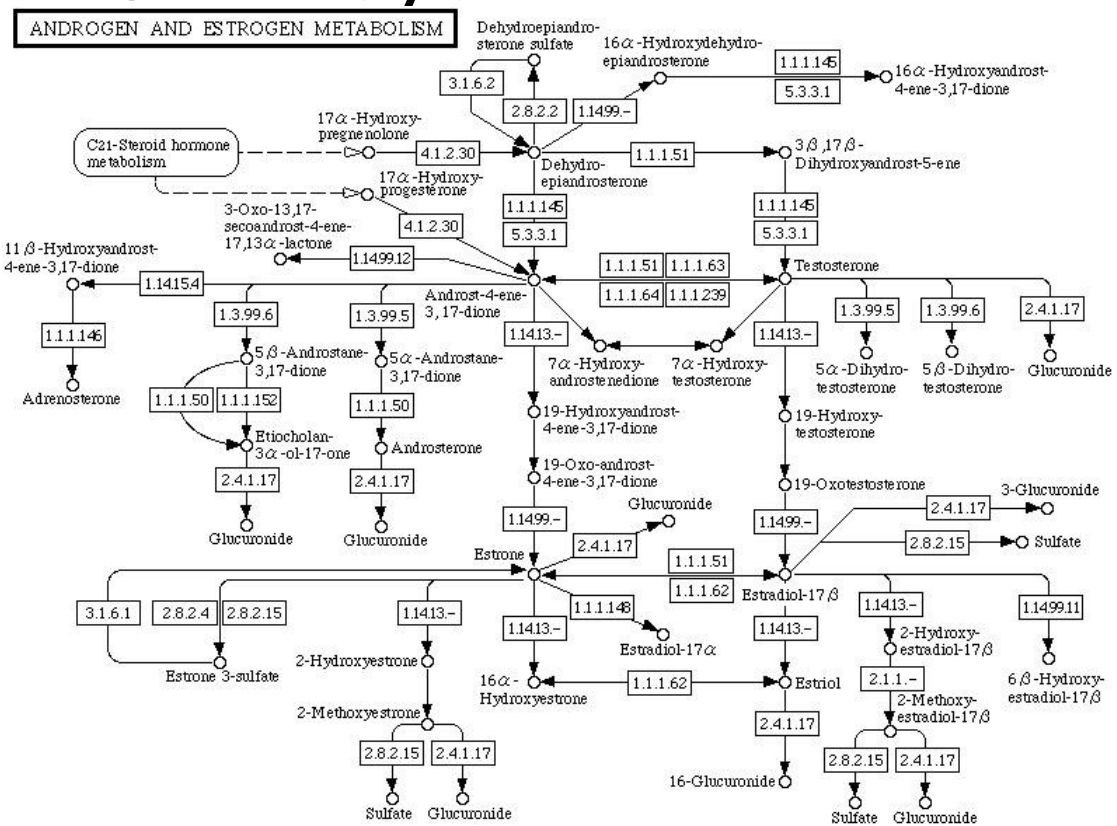


Genetic Engineering

- Modify the DNA blueprints
- Different machinery
- Different traits/behaviors

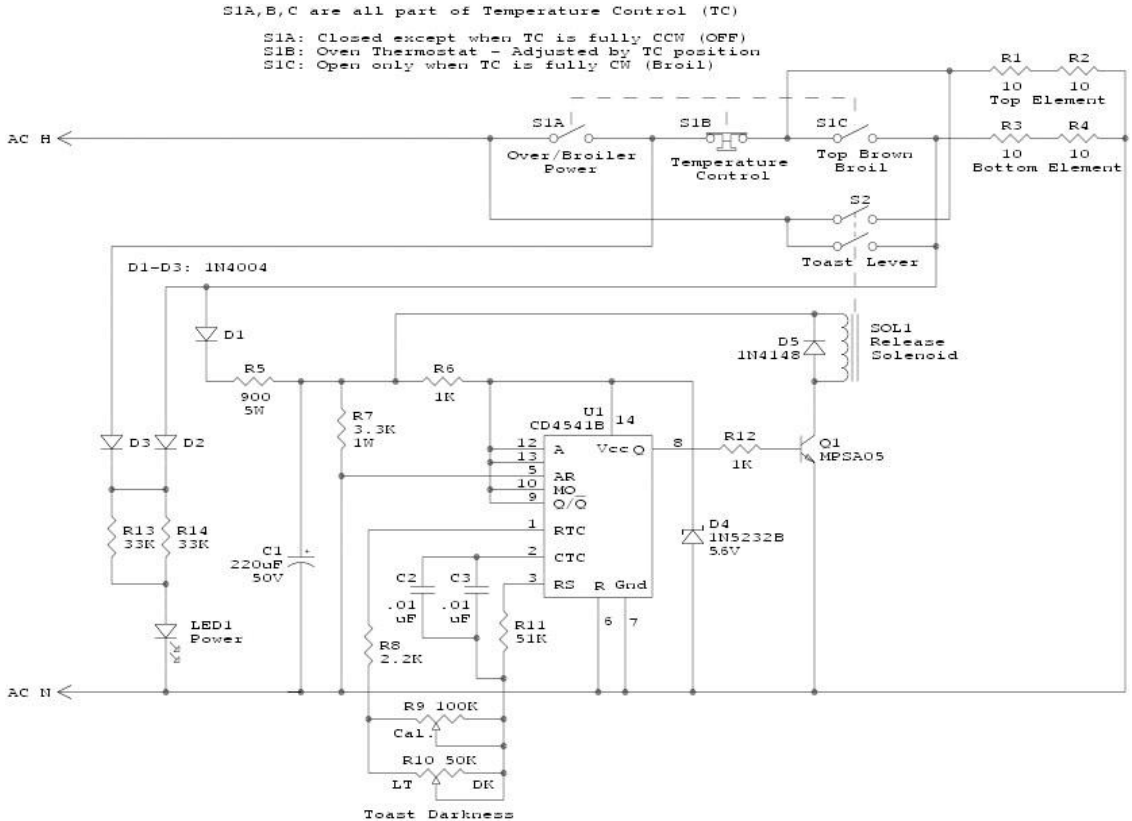
Increasing Complexity

- Cellular processes operate via an interwoven system



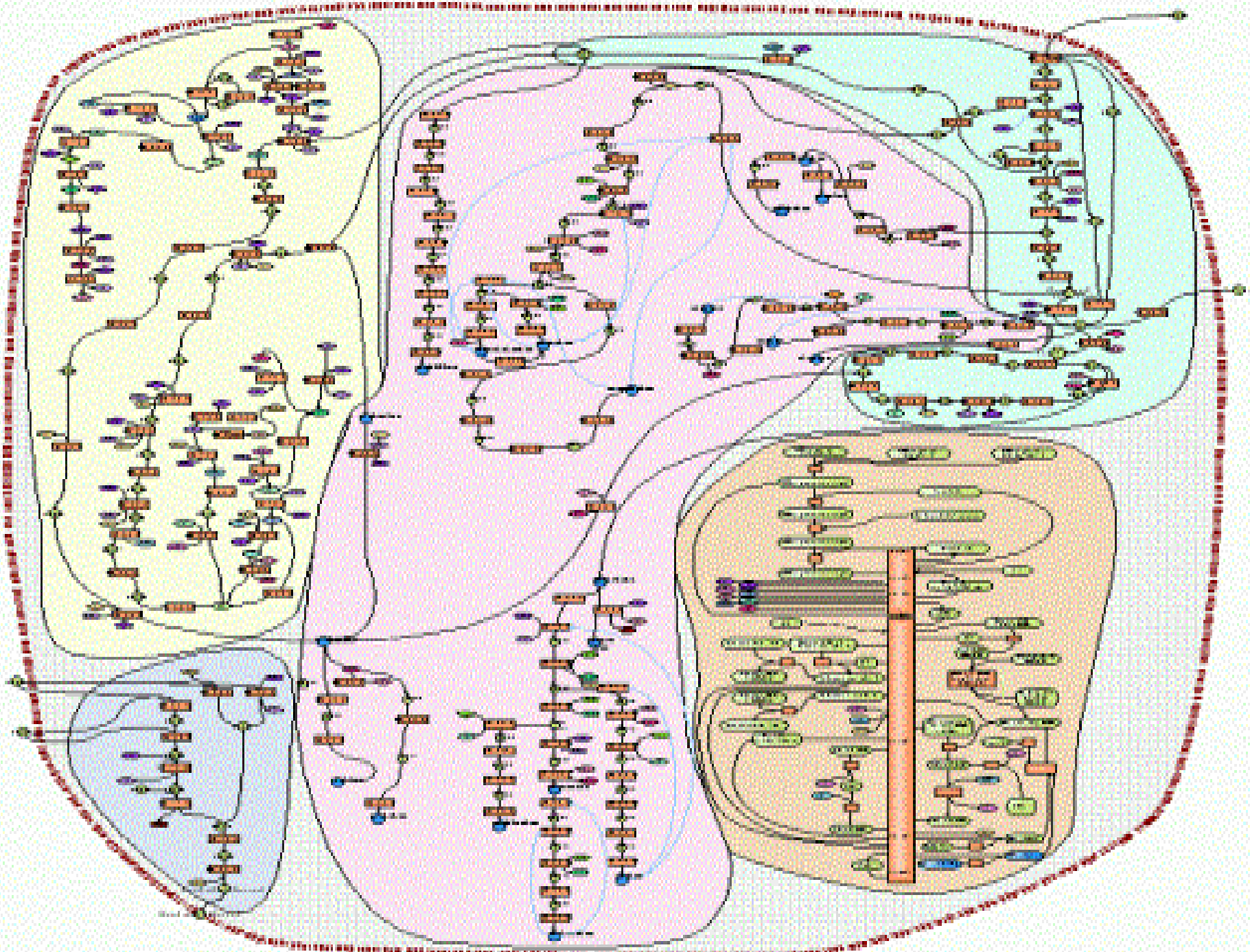
Increasing Complexity

- Can we make our own genetic "circuit"?



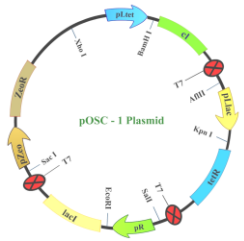
Project Description

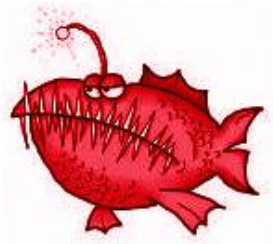
- Demonstrate a genetic circuit
- Model our genetic circuit
- Explore ethics and future possibilities



Objectives

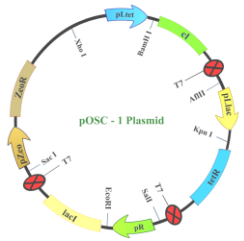
- Create non-natural engineered genetic circuit
- Place in higher organism
  - Port an already developed bacterial circuit to eukaryotes

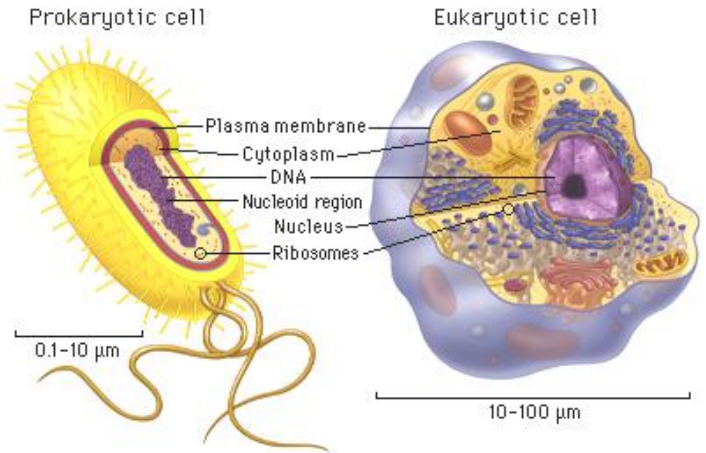




Issues

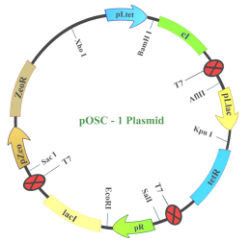
- Genetic components are not compatible between bacterial and eukaryotes
  - 'recode" our circuit
- Cannot interfere with host organism
  - We are not designing a whole organism, so we have to accommodate host



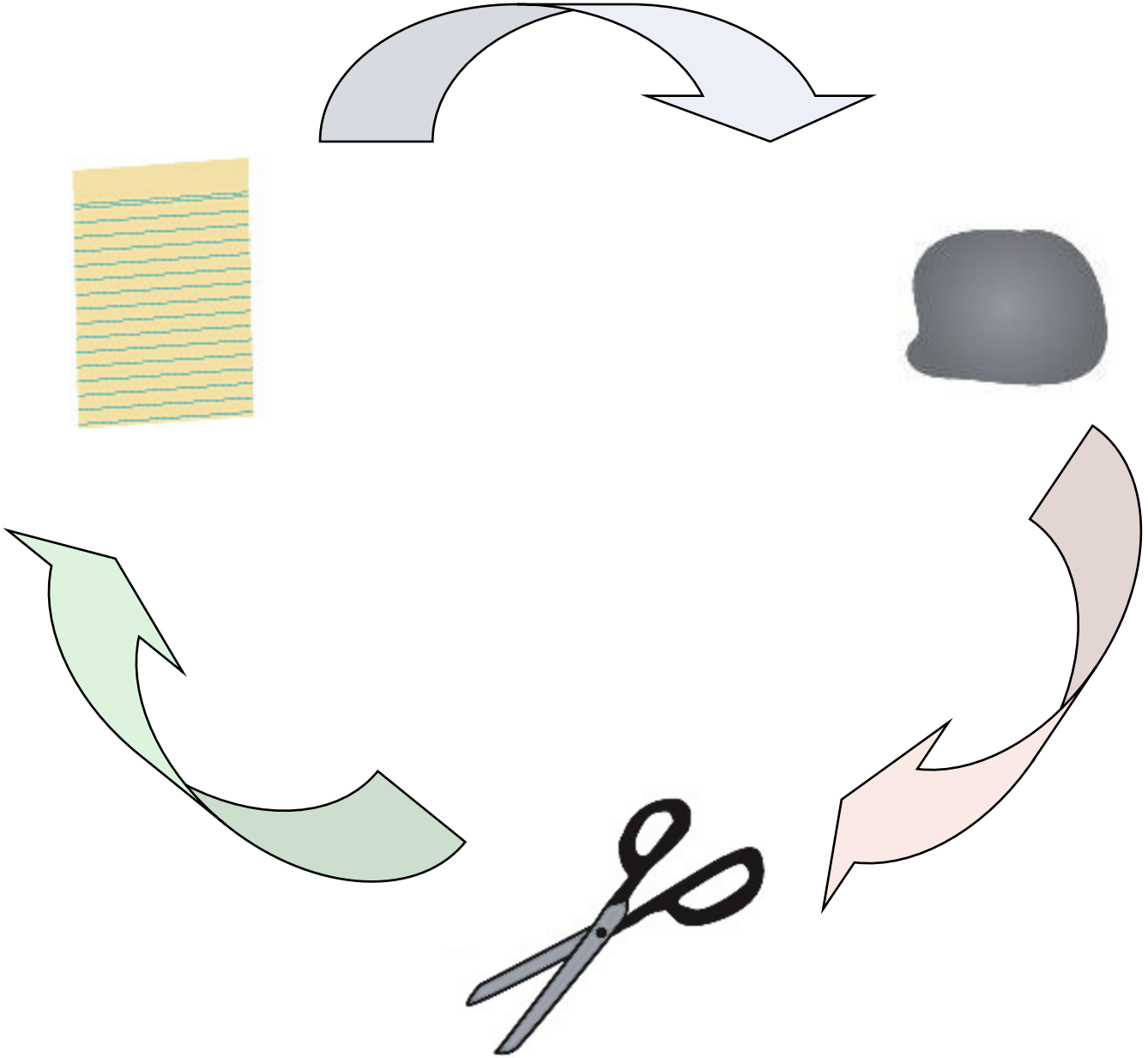


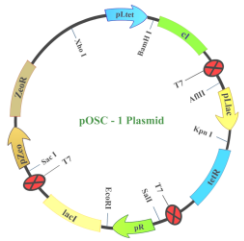
Designing the Circuit

- 3 genes and 3 promoters
- One gene and one promoter make one component of our system
- A self sustaining system



How the Oscillator Works





Planning the Oscillator

- Find the genes
- Define the primers
- Locate cutting sites

primers

- **CymR f**

```
                    5’-    atgagtccaa agagaagaac acag
1400 ccgctcgagt ctaggctagc atgagtccaa agagaagaac acaggcagag
       p  l  e   s  r  l   a  -  v  q   r  e  e   h  r  q   s
```

- pCymR f

5’-atgagtccaa agagaagaac acag Temp 54.7

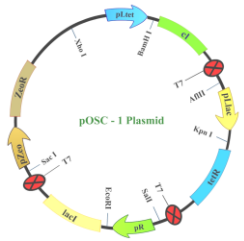
- **CymR r**

```
              5’-acgcgaaa ttcaagcgct ag
2000 cgcgagagcg gtacgcgaaa ttcaagcgct aggctagcct agagggcccg
     r  e  s   g  t  r   n  s  s  a   r  l  a   -  r  a   r  l
```

5’-acgcgaaa ttcaagcgct ag Tm 56.1

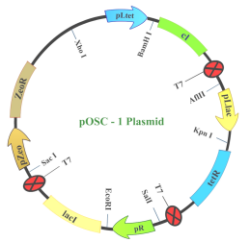
- pCymR r

Complement 5’-CTAGCGCTTGAATTTCGCGT Tm 56.1



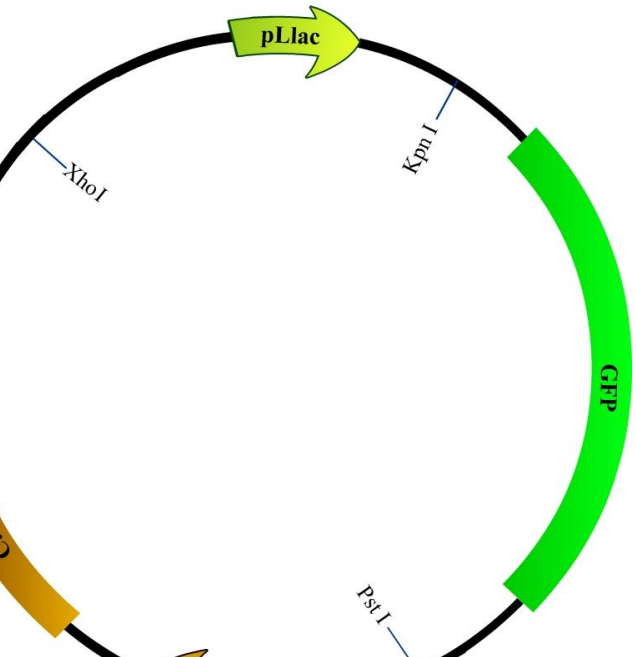
Detecting the Oscillations

- Three different reporters
- One for each gene
- Activates for that gene
- Three genes three colors



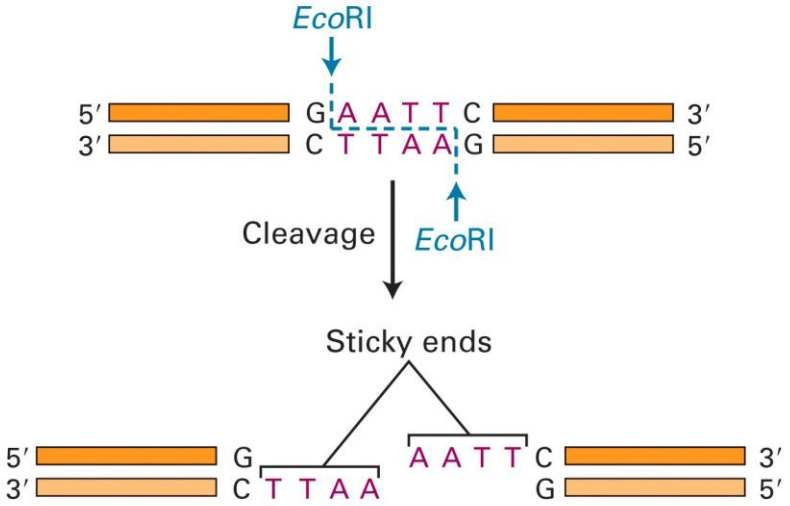
Constructing the Reporter

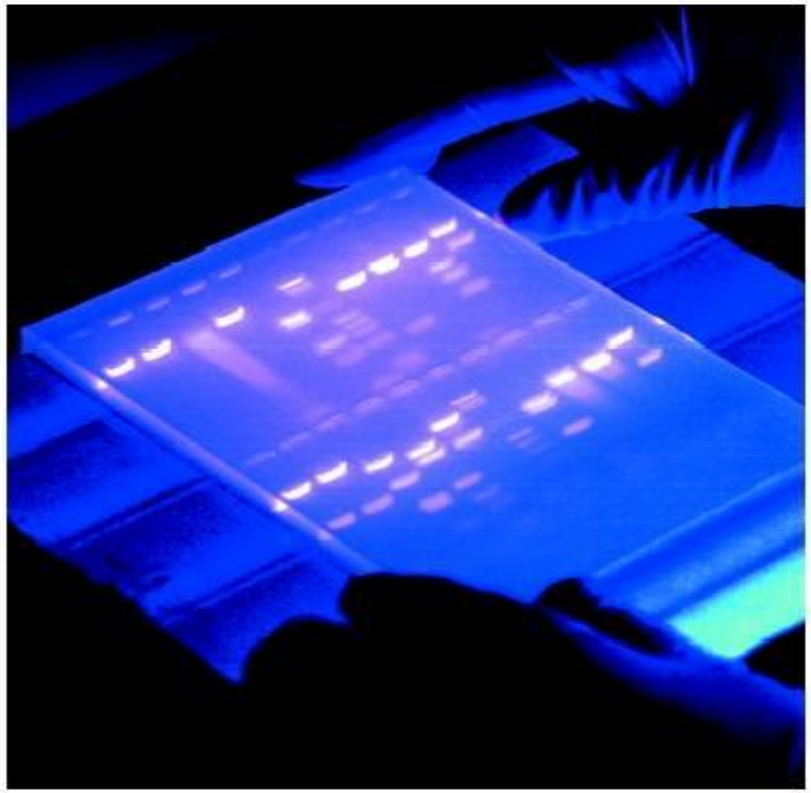
- How do we insert genes?
- Incompatibility between bacteria and fish cells?
- How do we assemble vectors?



Cut and Identify

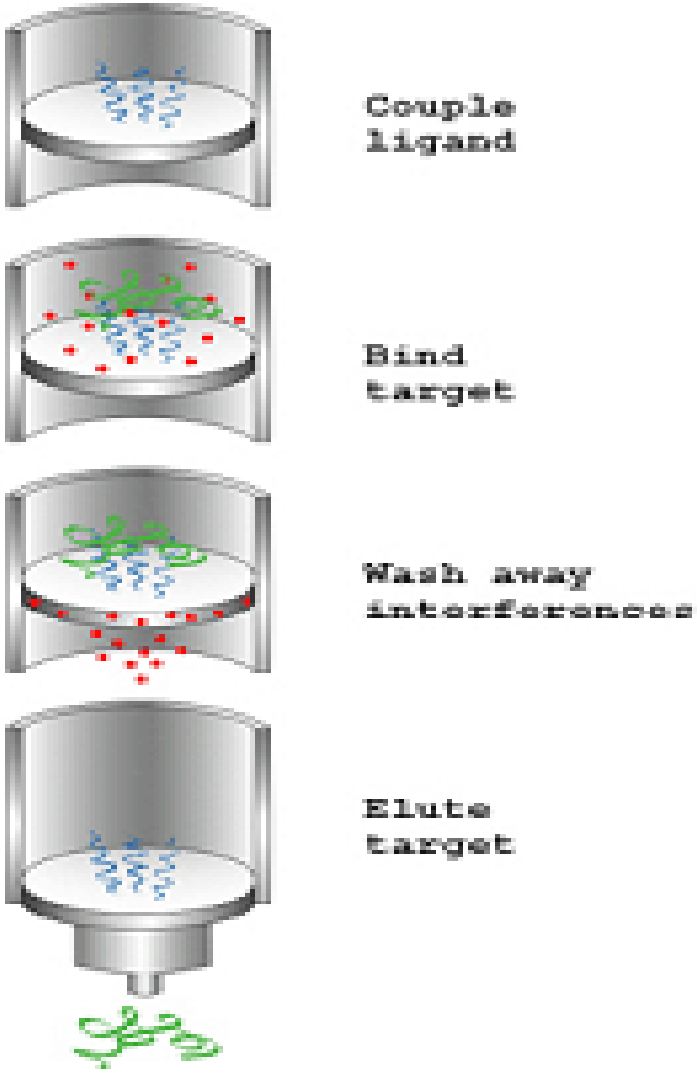
- Vector cut with known restriction enzyme
- DNA separation by weight via gel electrophoresis





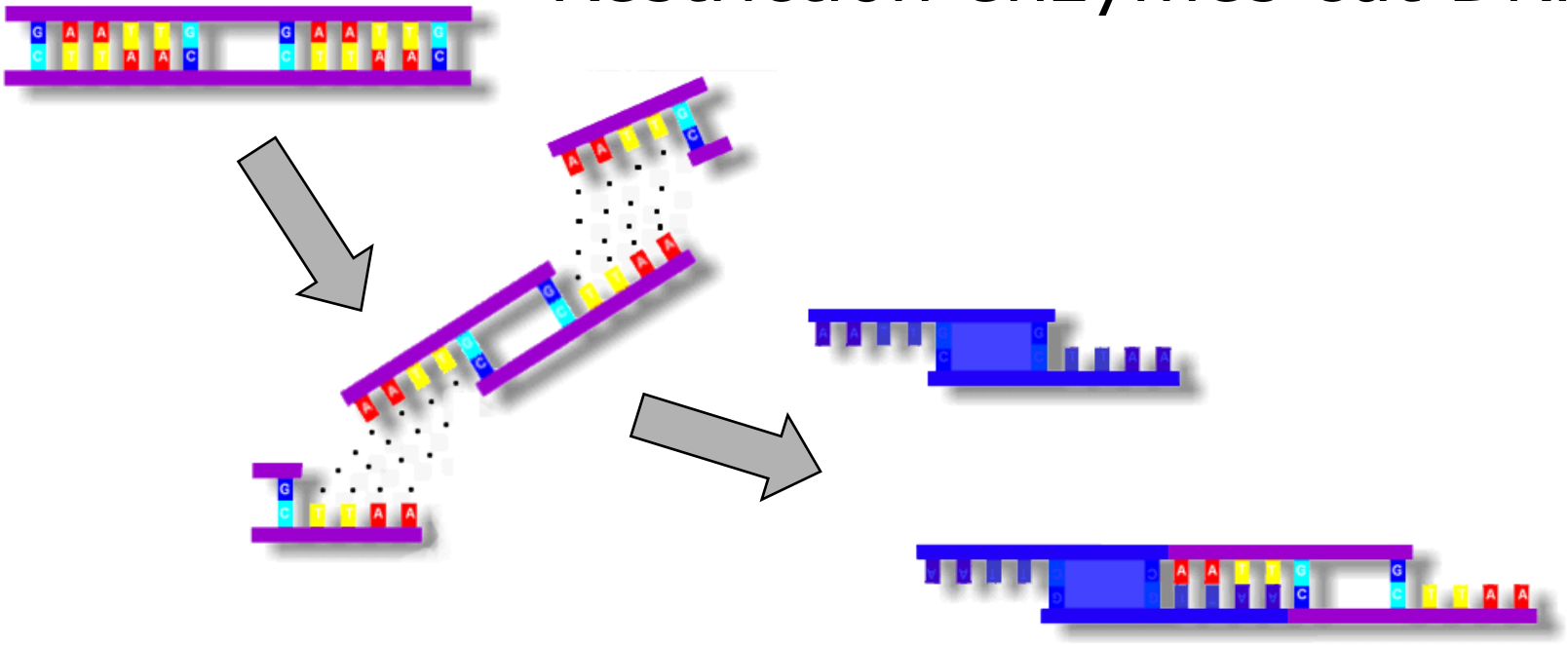
Extract and Purify

- Gel is cut and filtered to extract



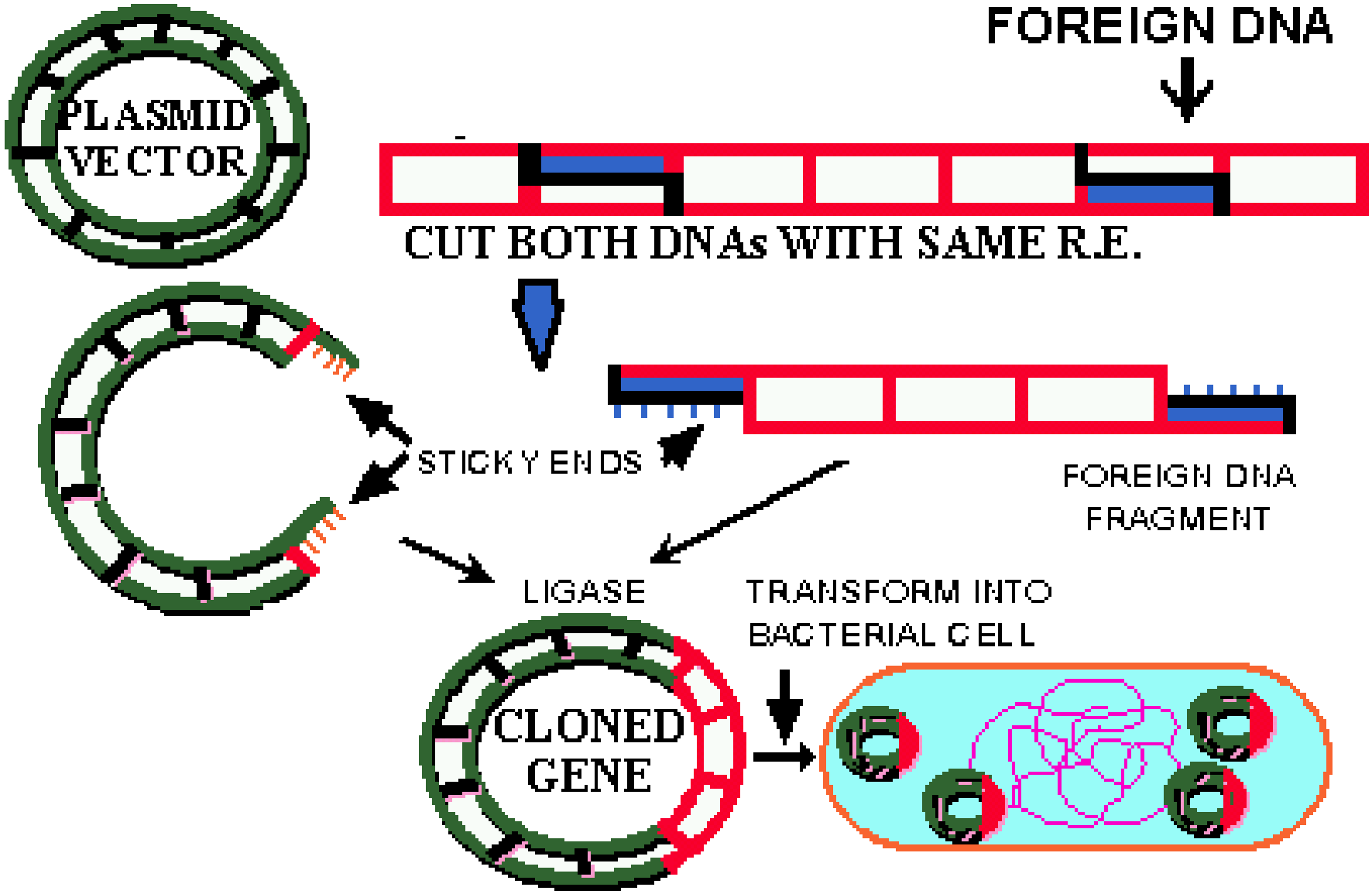
Ligation

Restriction enzymes cut DNA



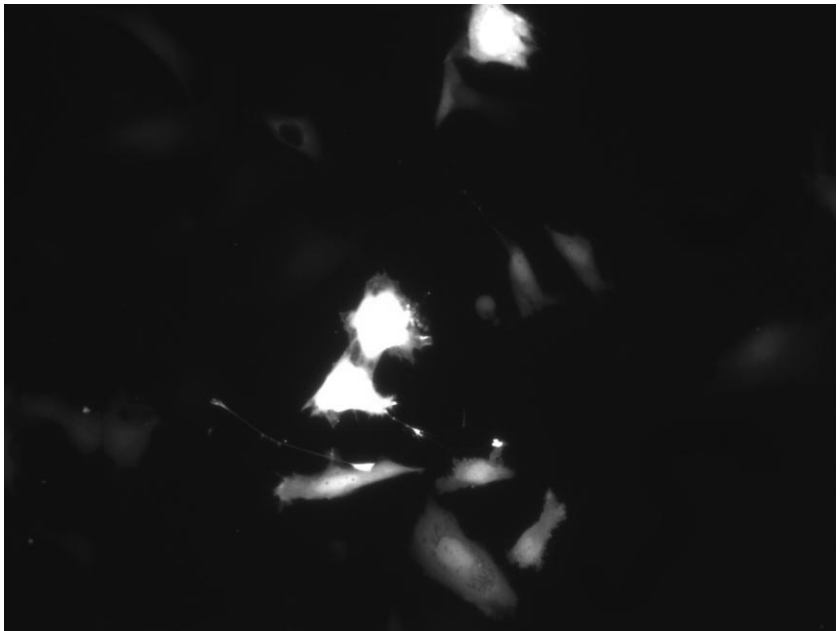
Ligase binds DNA pieces

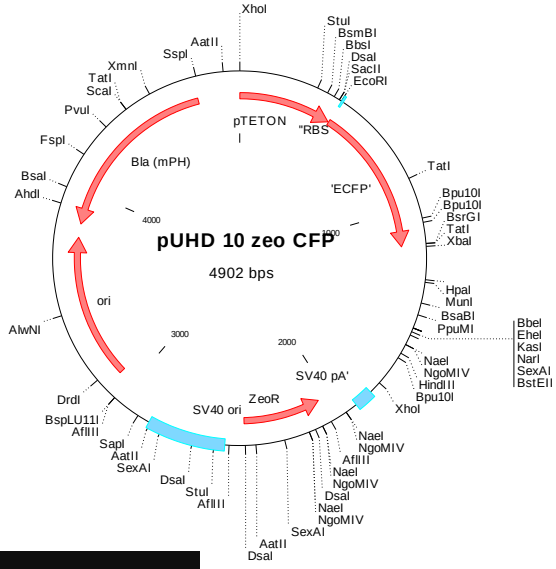
Overview

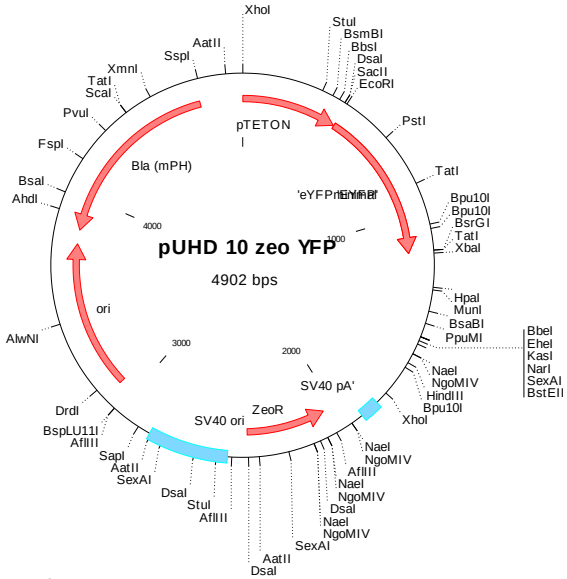

PLASMID VECTOR
FOREIGN DNA
CUT BOTH DNAs WITH SAME R.E.
STICKY ENDS
FOREIGN DNA FRAGMENT
LIGASE
TRANSFORM INTO BACTERIAL CELL
CLONED GENE

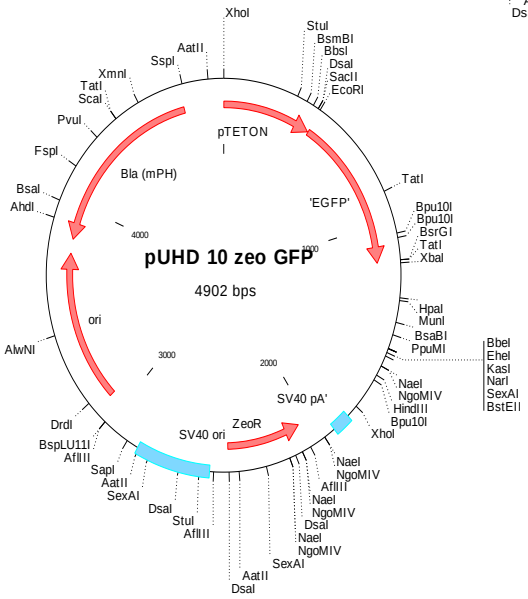
Finished Product

- 3 reporter plasmids
- Indicators for use in fish cells









Zebra Fish

Danio rerio

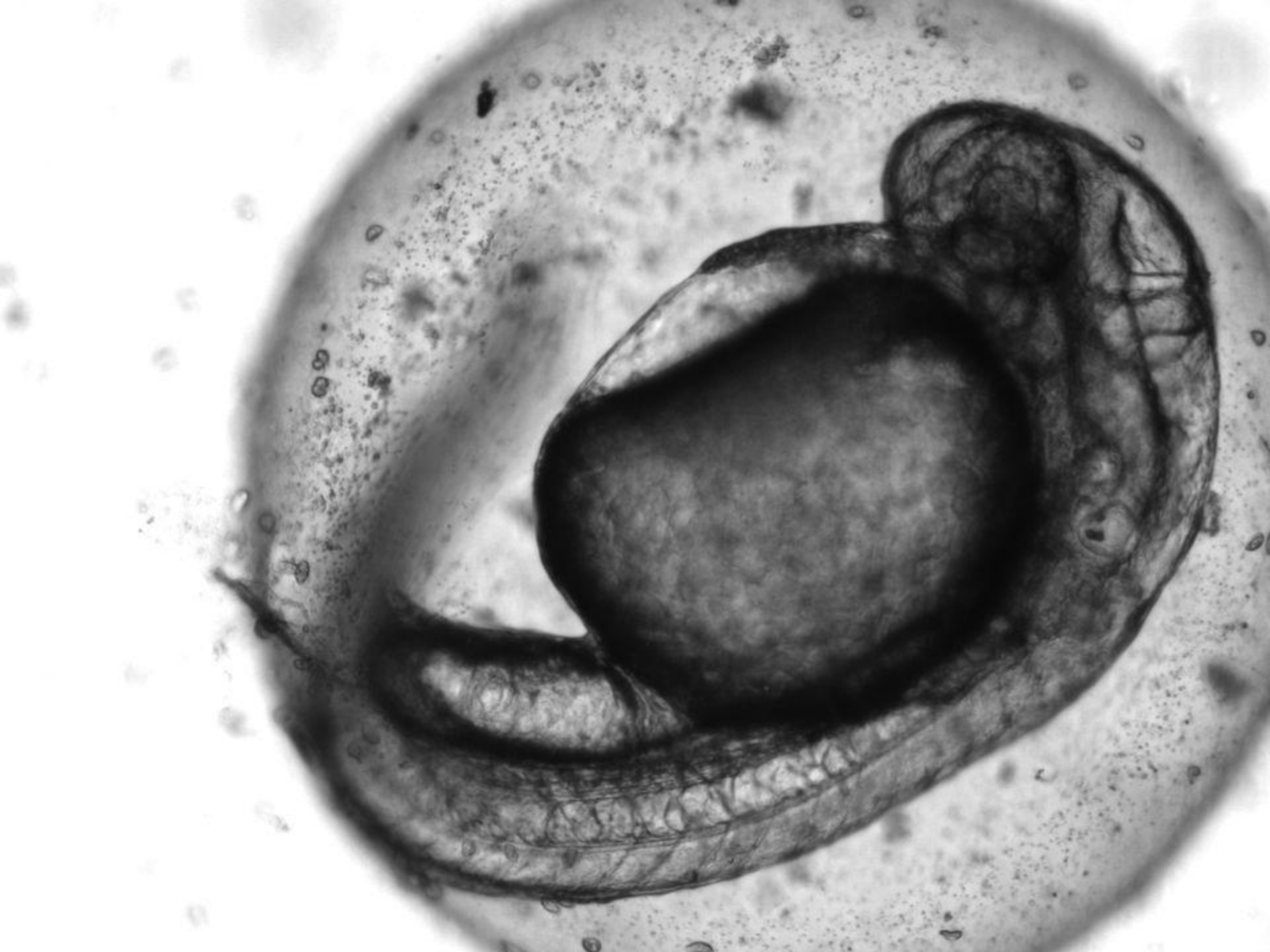
- Easy to maintain
- Easy to breed
- Quick developmental cycle
- Transparent embryonic growth

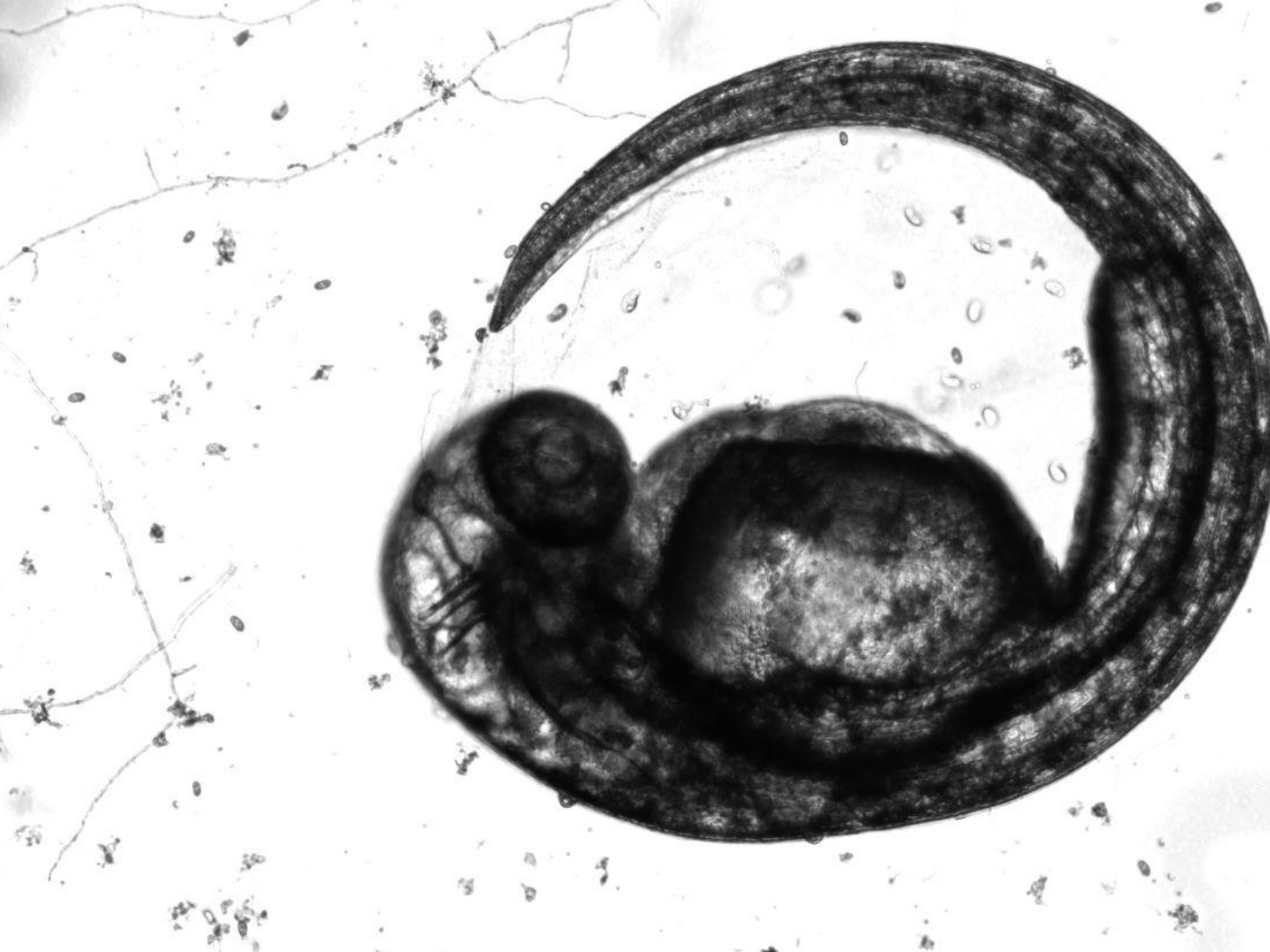
Courtesy of Aquahabitats.com

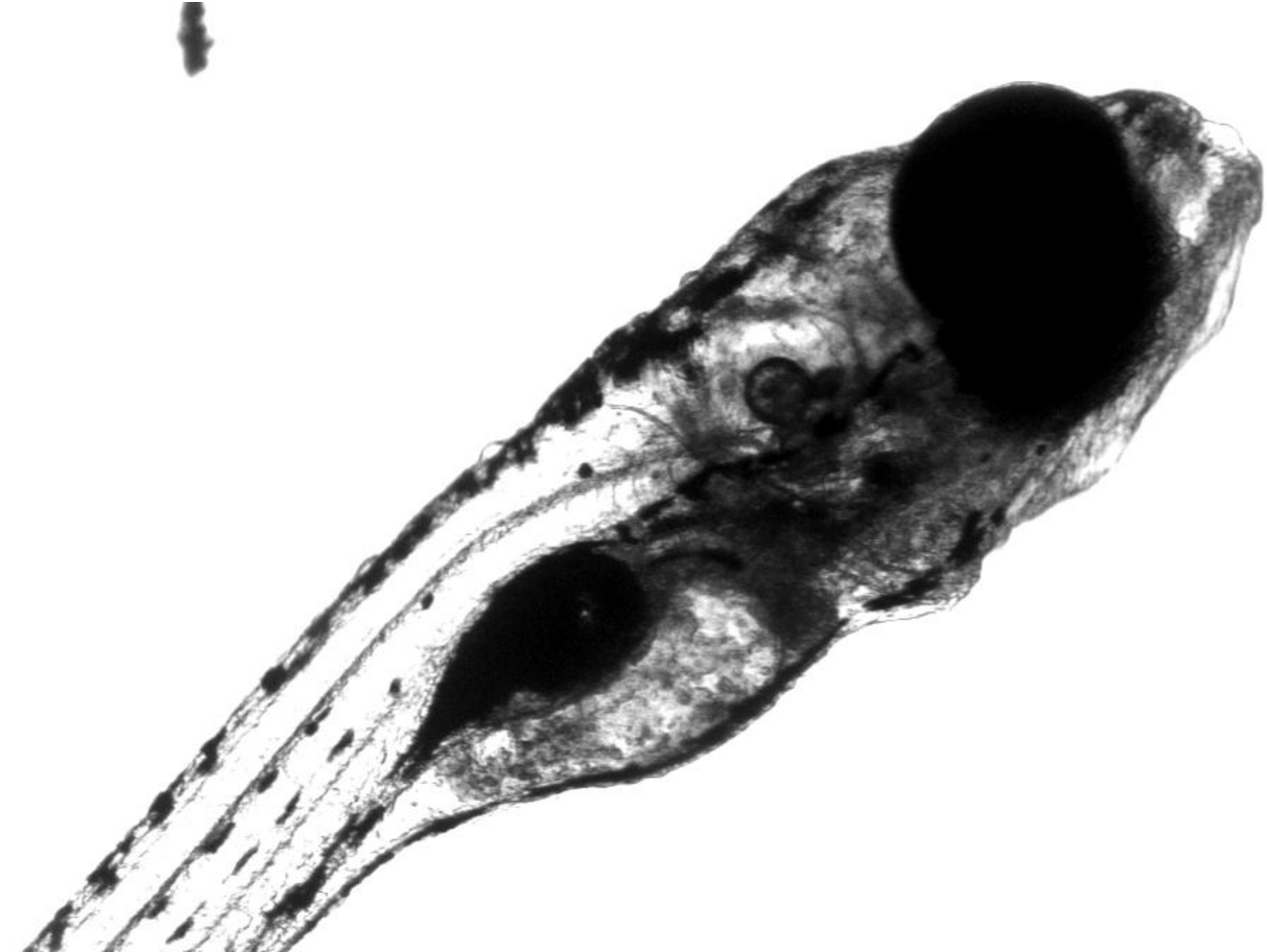
Encountered Problems

- Feeding and cleaning
- Harnessing the eggs
- Gene insertion

Courtesy of Aquahabitats.com

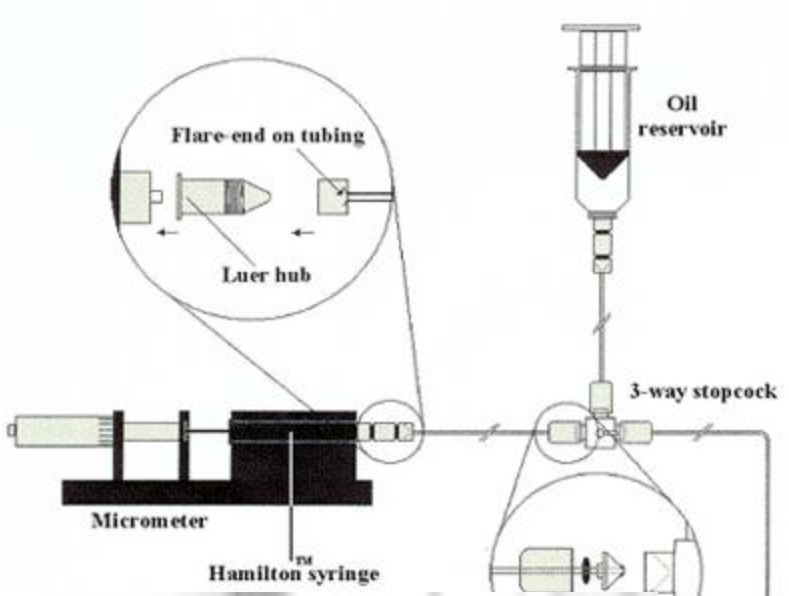


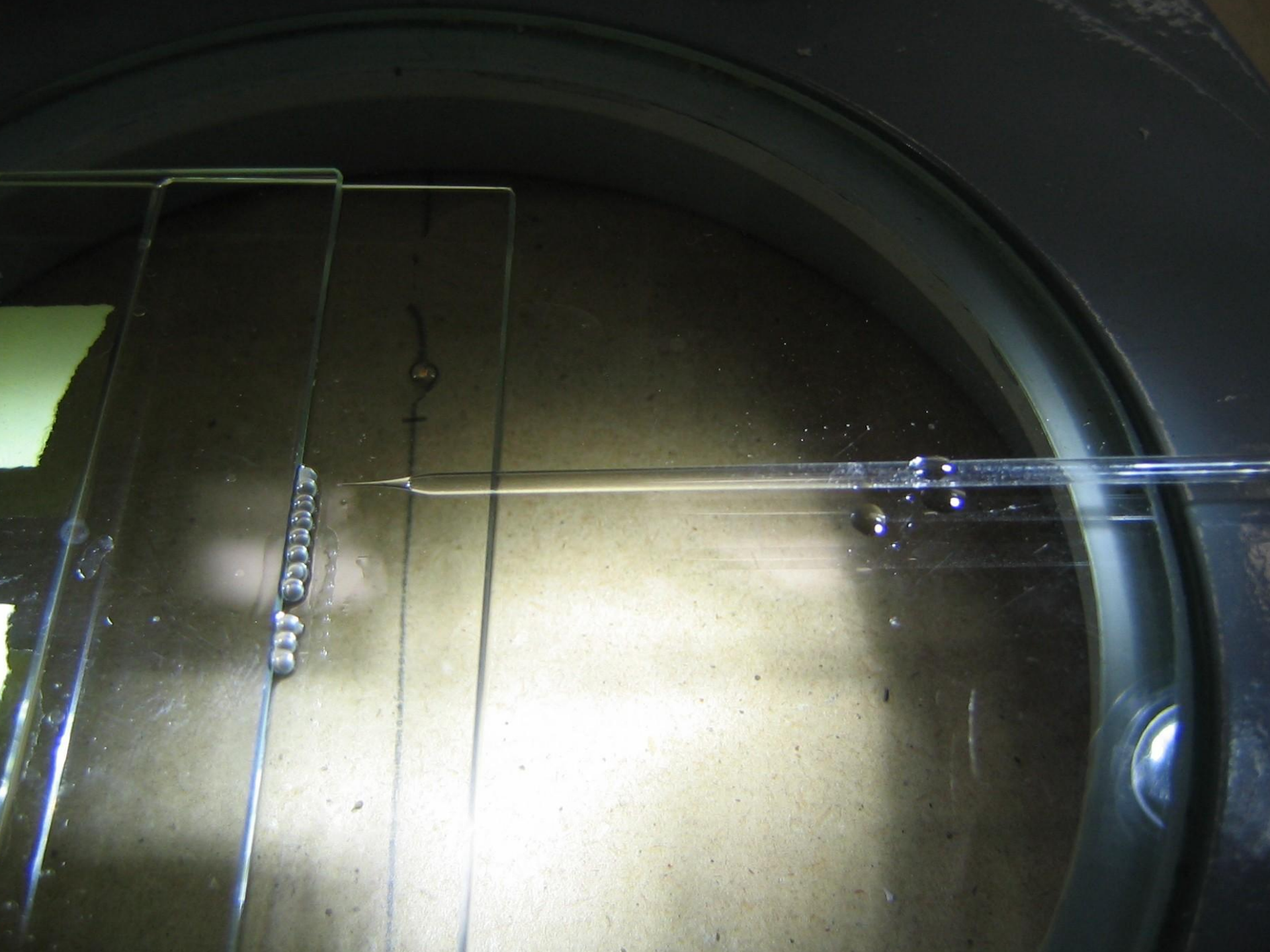


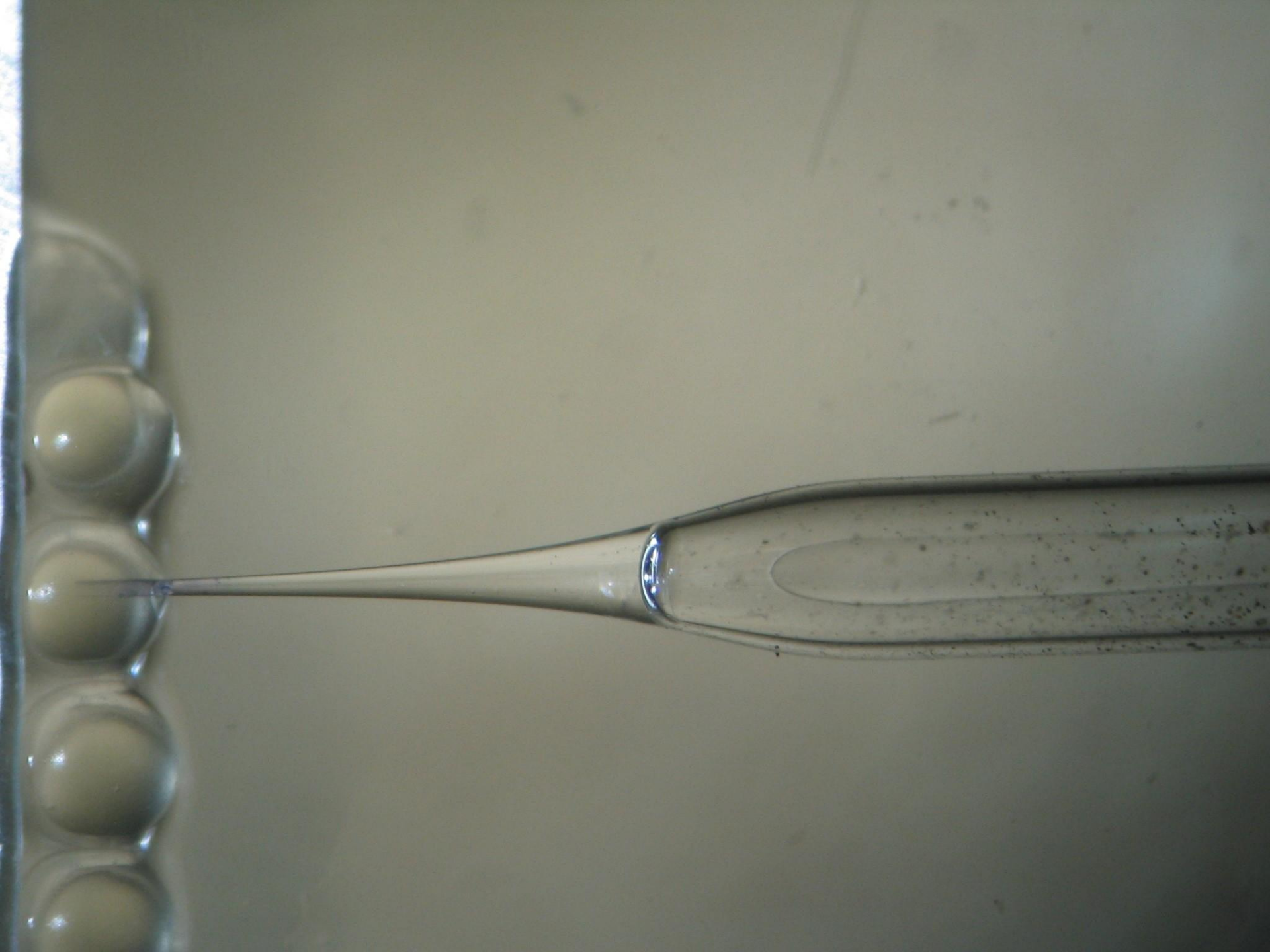


Micro Injection

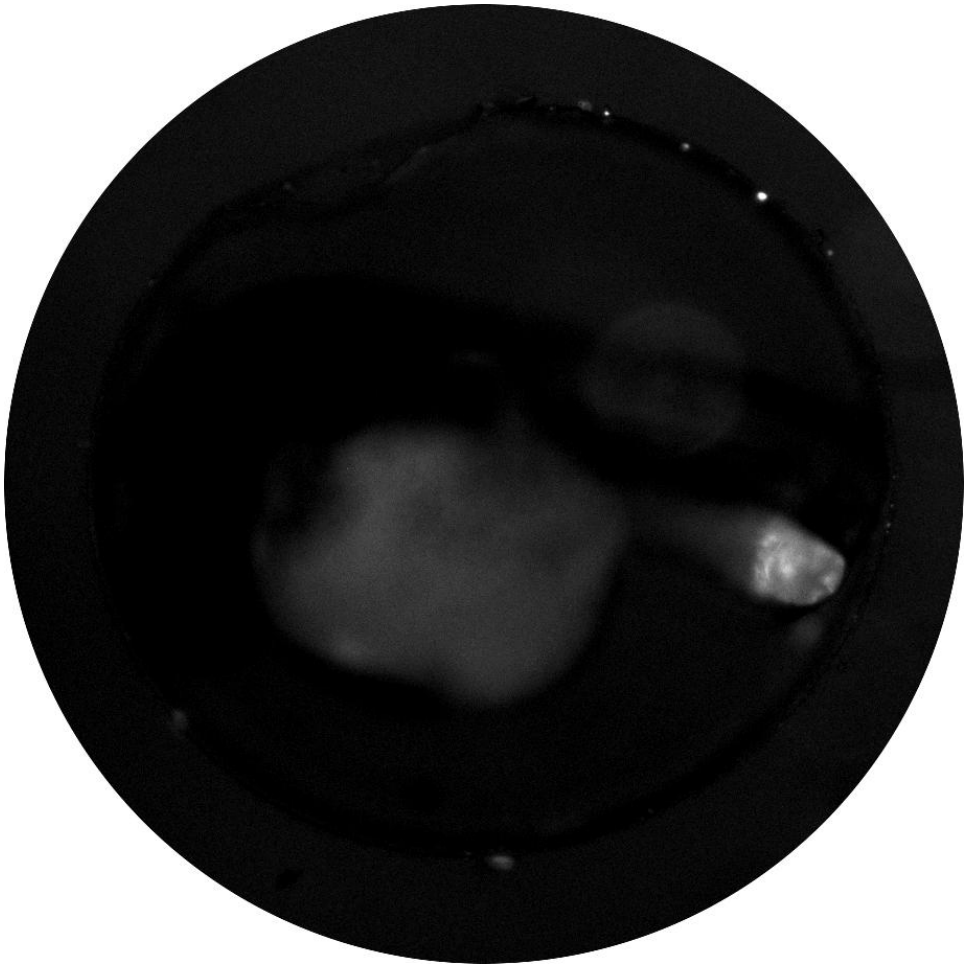
- The idea
- The set-up
- The process







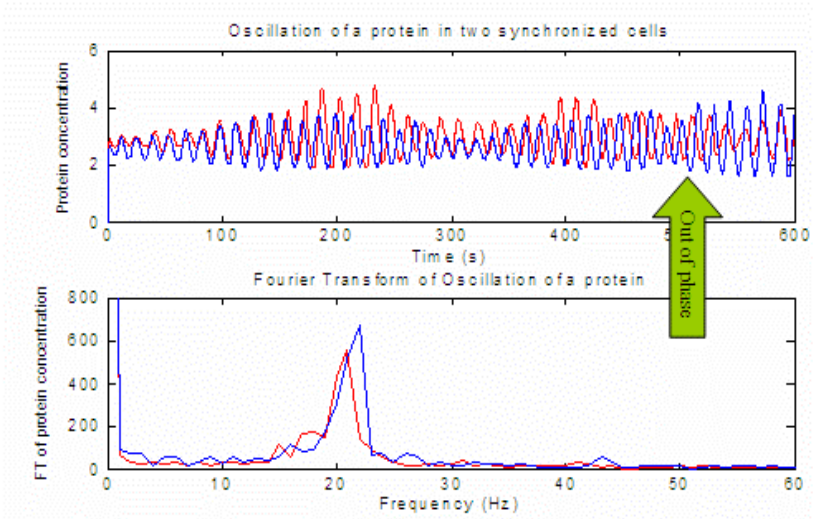
Brightfield image

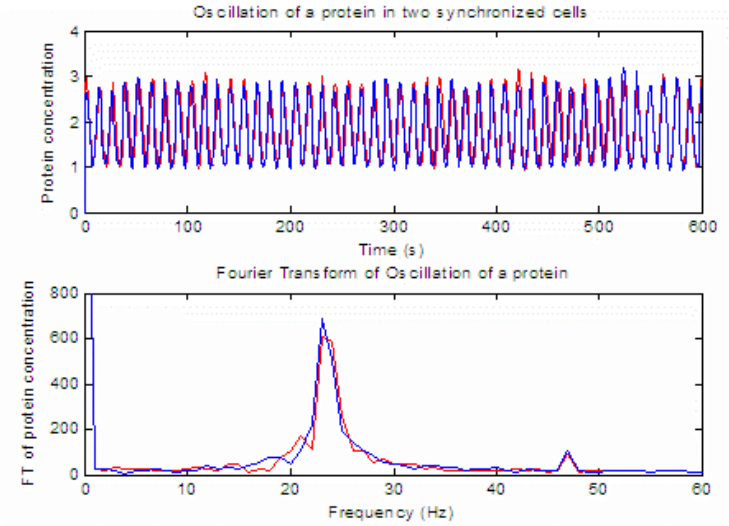


Fluorescence image

Math Modelling

- Synchronization
  - Within Individual Cells
  - Multicellular System
  - Whole Organism





Simulation

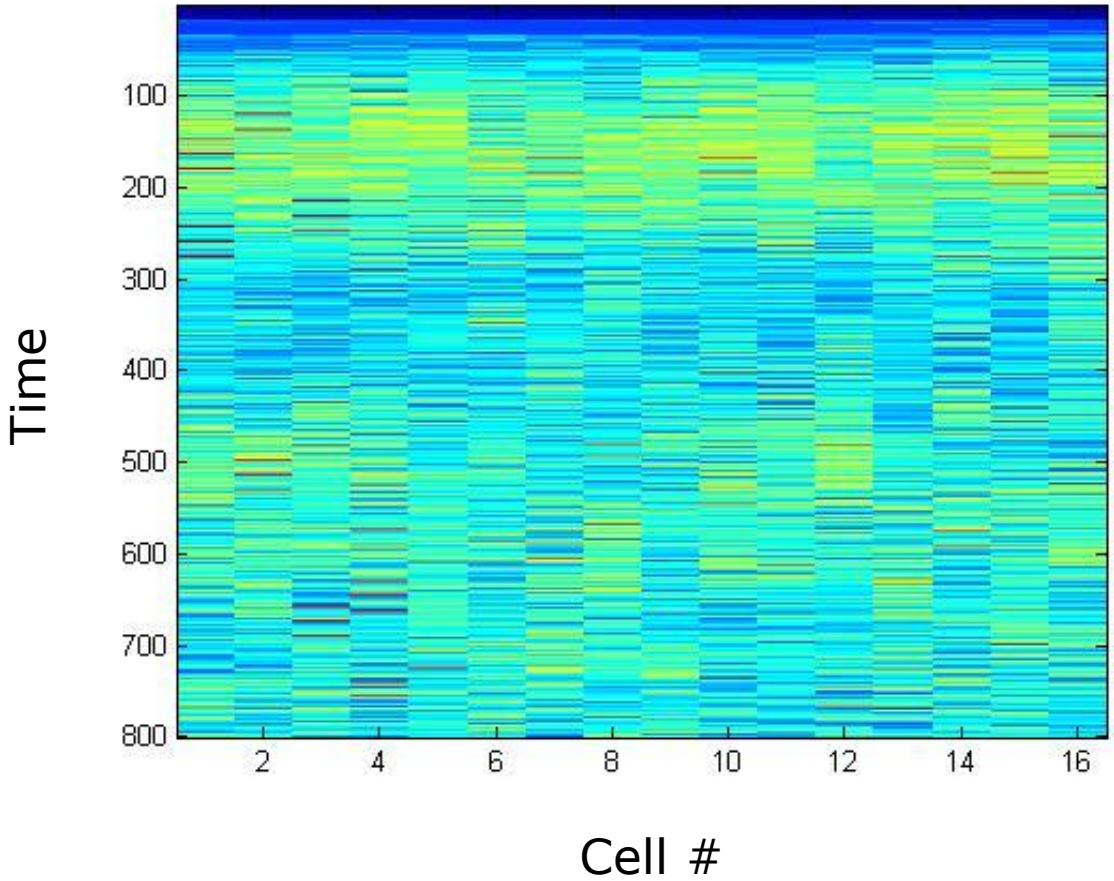
- Biology is messy
  - Genes are ‘messier’ than switches
  - No simple ON/OFF
  - Randomness
- Differential Equation System
- Optimization

- C++ and MATLAB
- Co-operation
- Interpretation

```
{
for (int i=0;i<5;i++)
{
if (time==0)
initcells();
else
resetprot(prot[0], prot[1], prot[2], mRNA[0], mRNA[1], mRNA[2], test);

switch(m)
{default:
case DETERMINISTIC:
mRNA[0] = mRNA[0] + (a0[2] + a1[2]*pow(kdsynch, 2.5)/(pow(kdsynch,
2.5)+pow(prot[2], 2.5)) - br*mRNA[0])*h;

prot[0] = prot[0] + (ts*mRNA[0] - bp*prot[0])*h;

mRNA[1] = mRNA[1] + (a0[0] + a1[0]*pow(kdsynch, 2.5)/(pow(kdsynch,
2.5)+pow(prot[0], 2.5)) - br*mRNA[1])*h;

prot[1] = prot[1] + (ts*mRNA[1] - bp*prot[1])*h;

mRNA[2] = mRNA[2] + (a0[1] + a1[1]*pow( kdsynch, 2.5)/(pow(kdsynch,
2.5)+pow(prot[1], 2.5)) - br*mRNA[2])*h;

prot[2] = prot[2] + (ts*mRNA[2] - bp*prot[2])*h;
break;
}
```

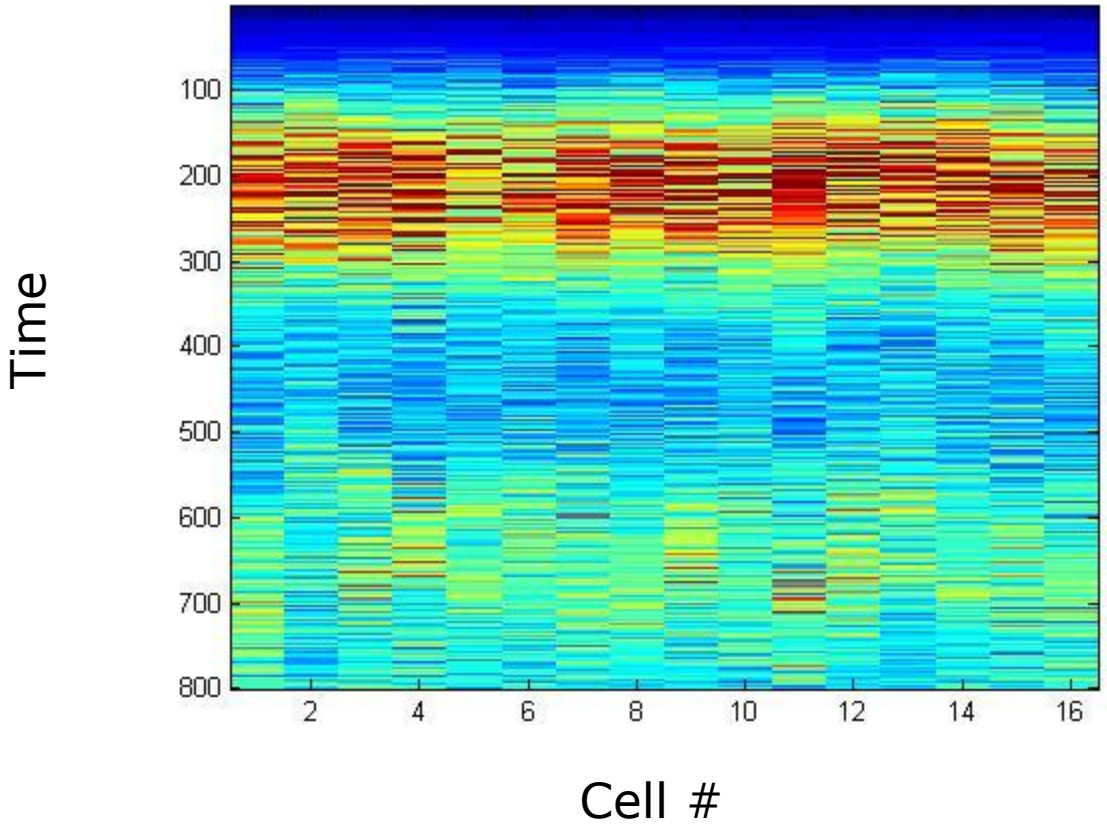
Unsynchronized

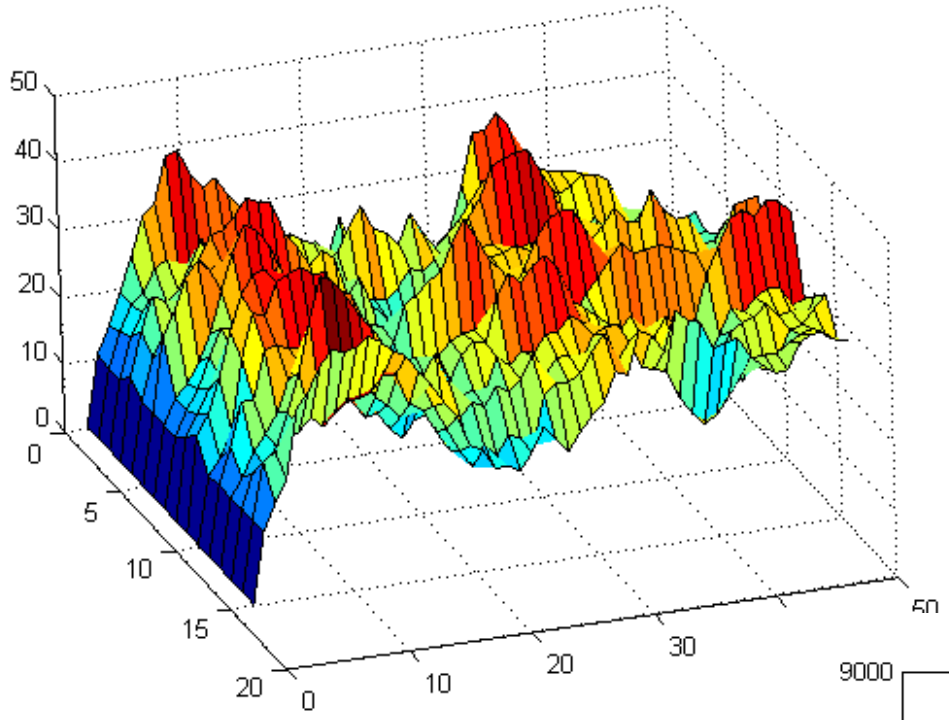
- Tracks whether or not one of the genes is ‘turned on’ in a given cell at a given time



Synchronized

- Tracks whether or not one of the genes is ‘turned on’ in a given cell at a given time

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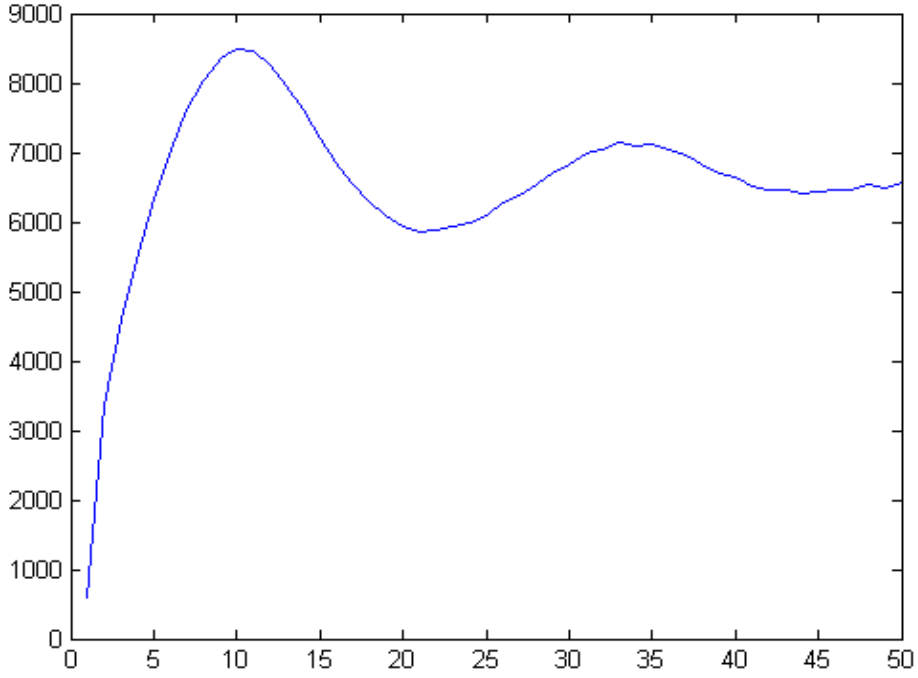
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6000
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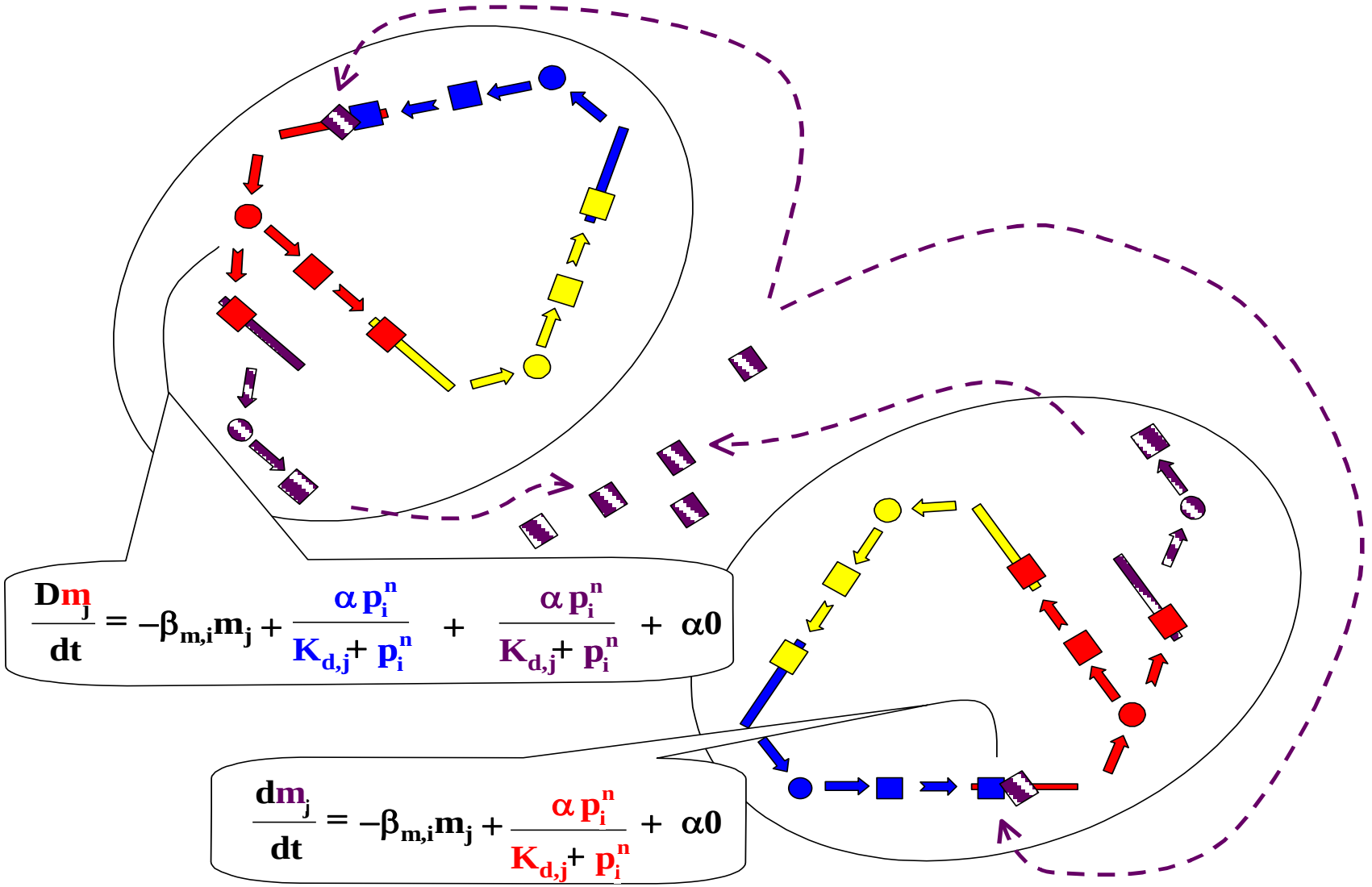
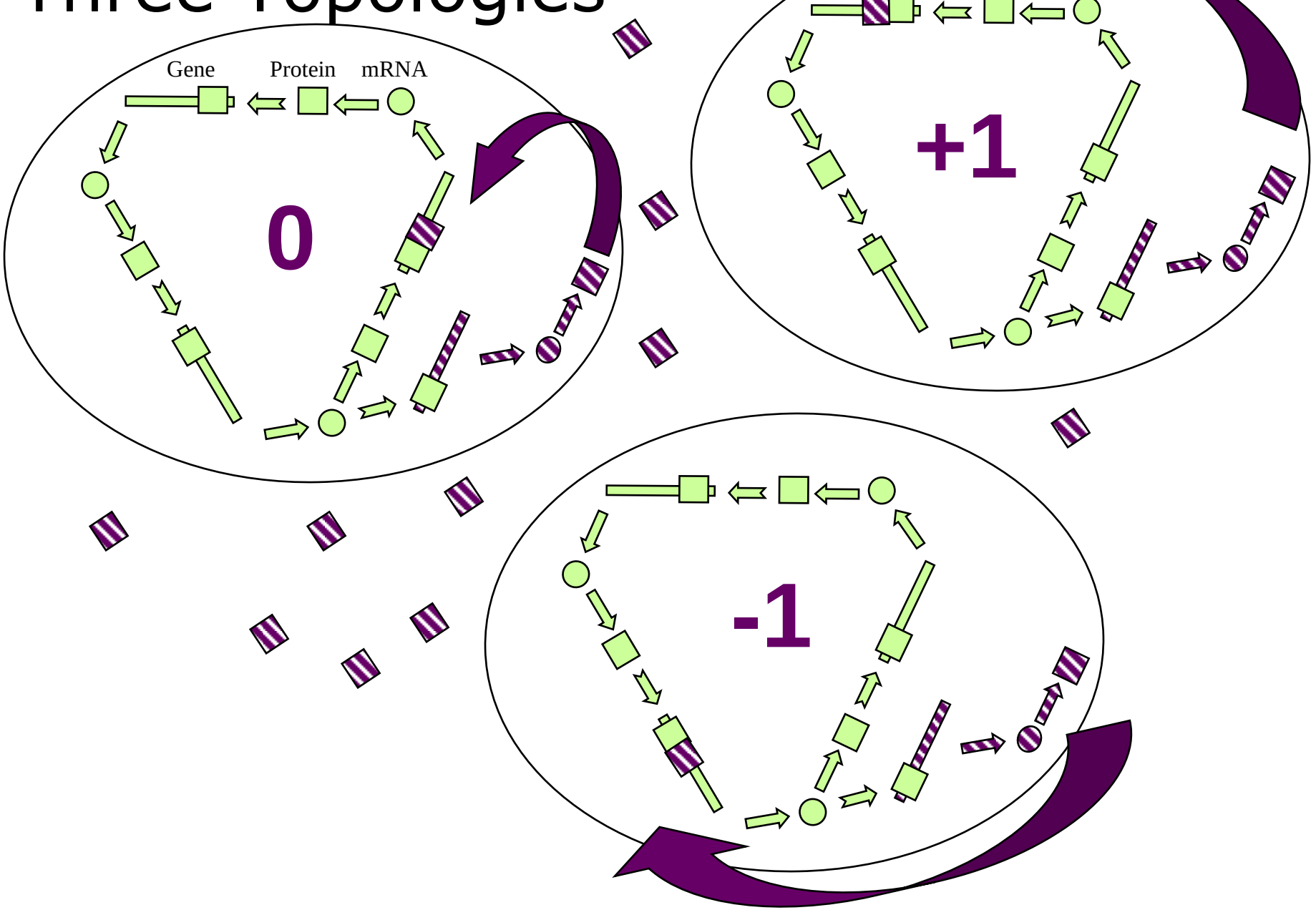
Synchronization

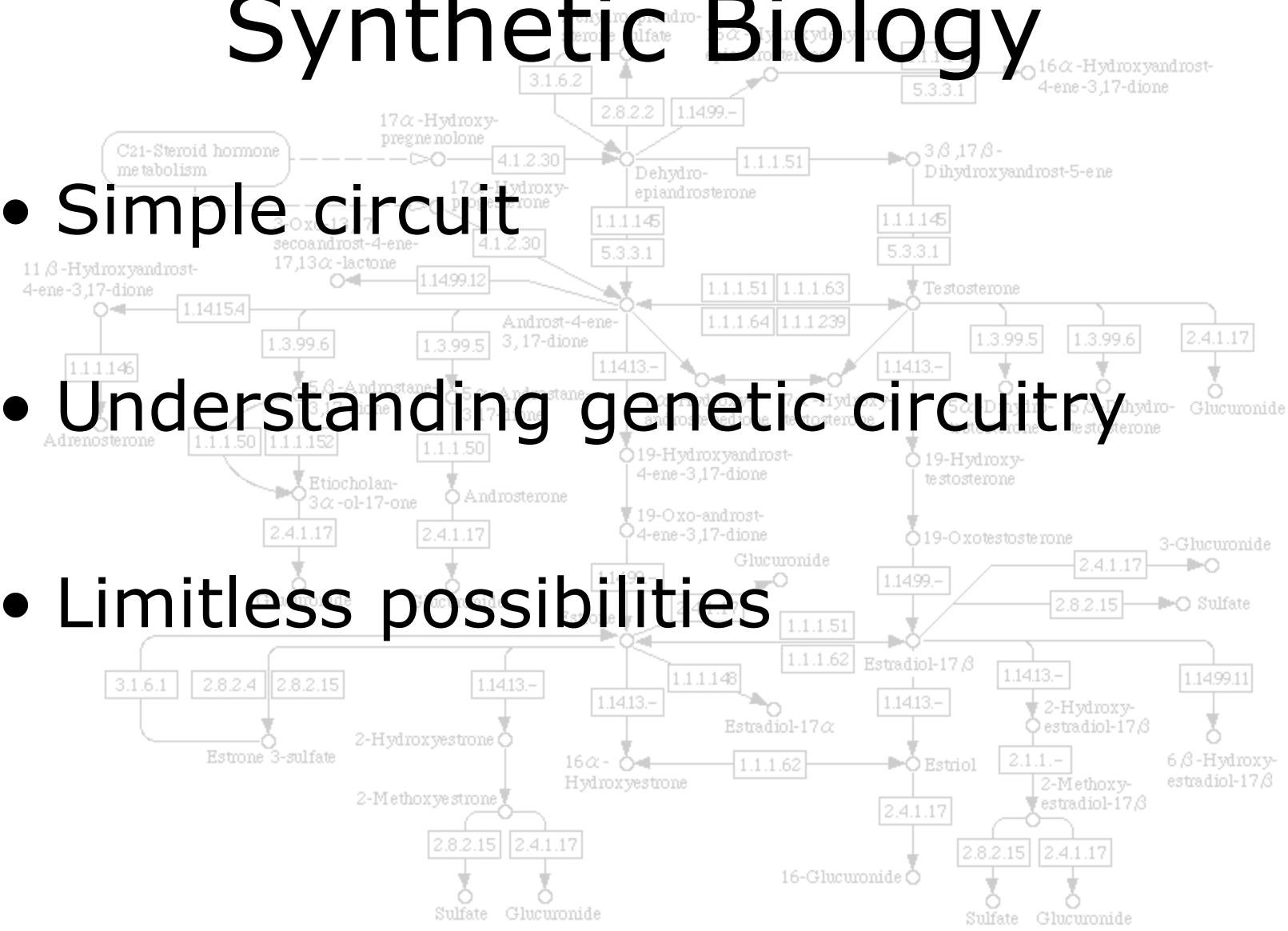


Three Topologies



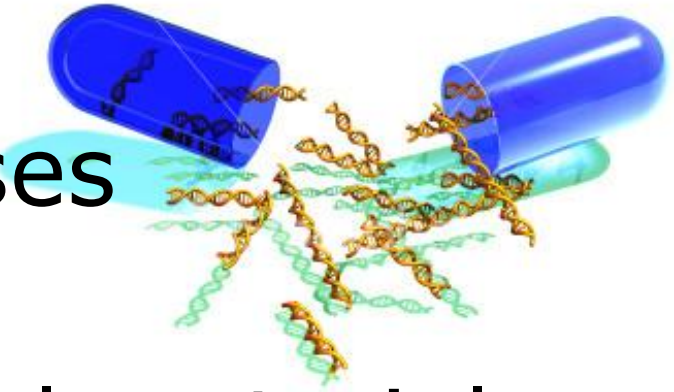
Synthetic Biology

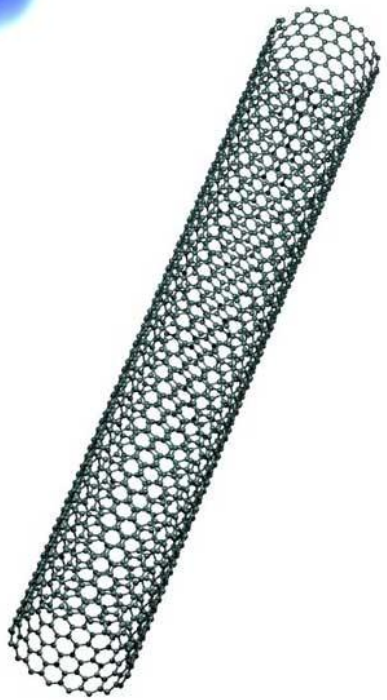
- Simple circuit
- Understanding genetic circuitry
- Limitless possibilities

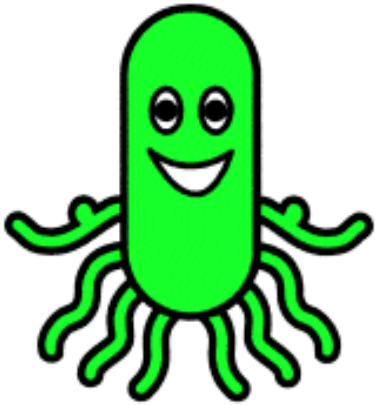


Improve life quality

- Curing diseases
- Creating novel materials
- Bioremediation







The Possibilities

Modifying genomes

?

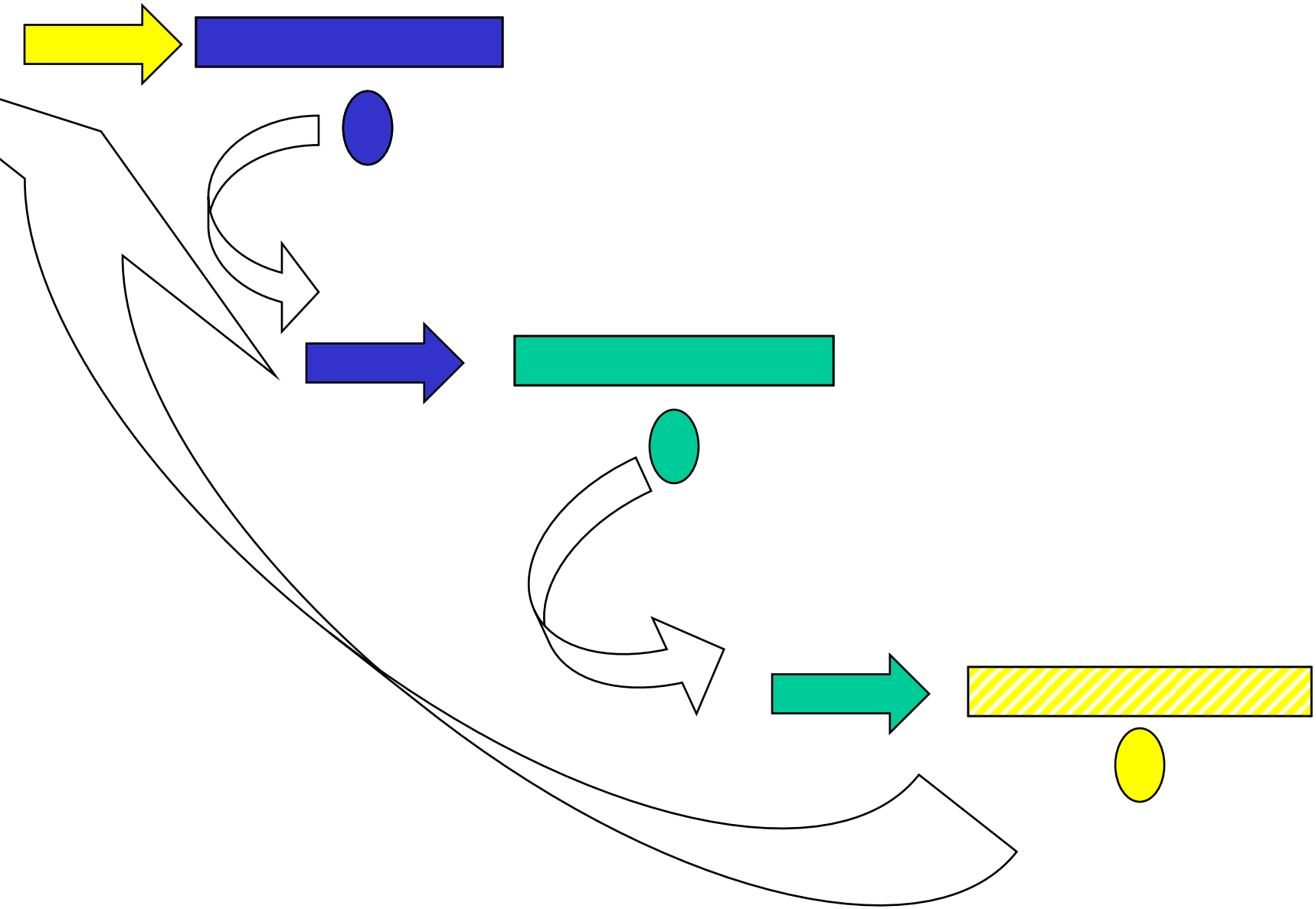
Governing evolution

What is life?

Creating life

But what is the future?

How The Oscillator Works



How The Oscillator Works