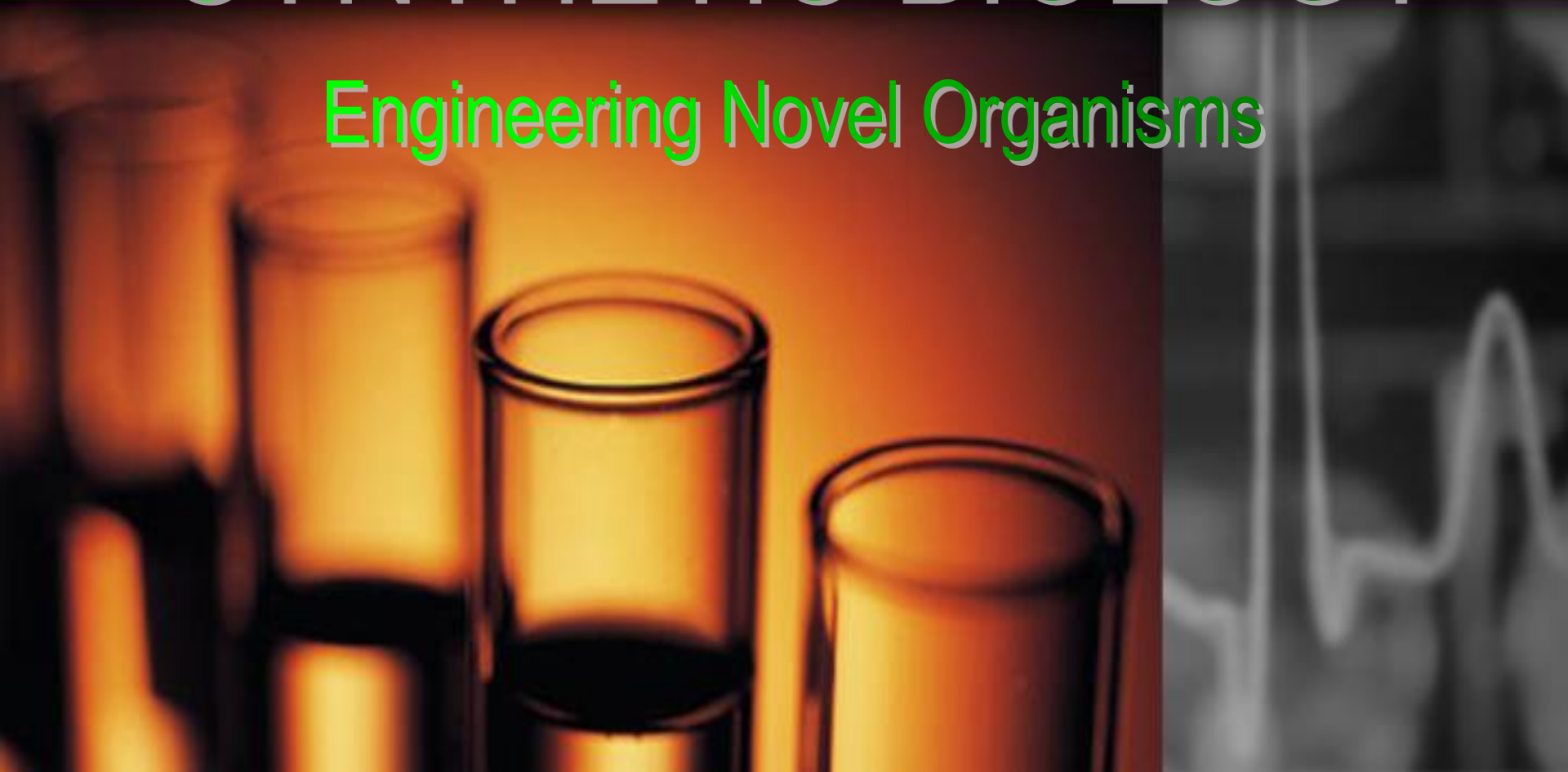


IPRO 302

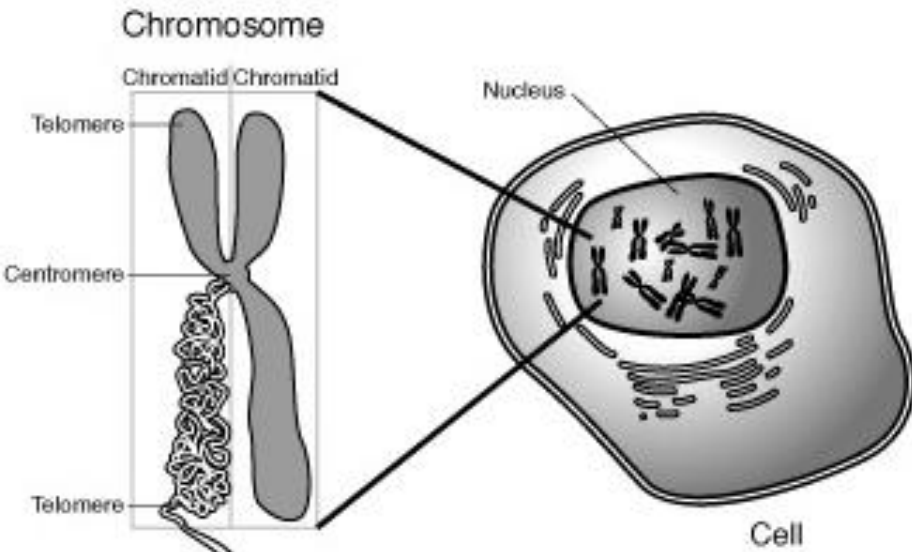
SYNTHETIC BIOLOGY

Engineering Novel Organisms



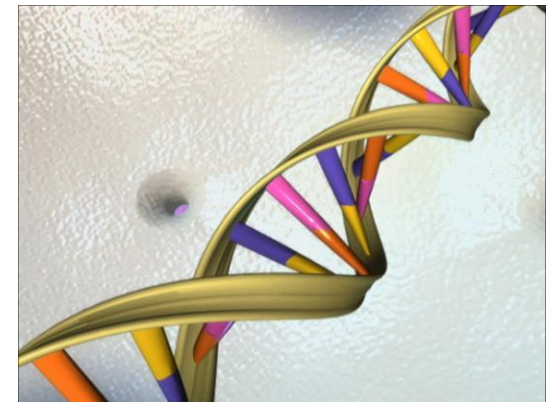
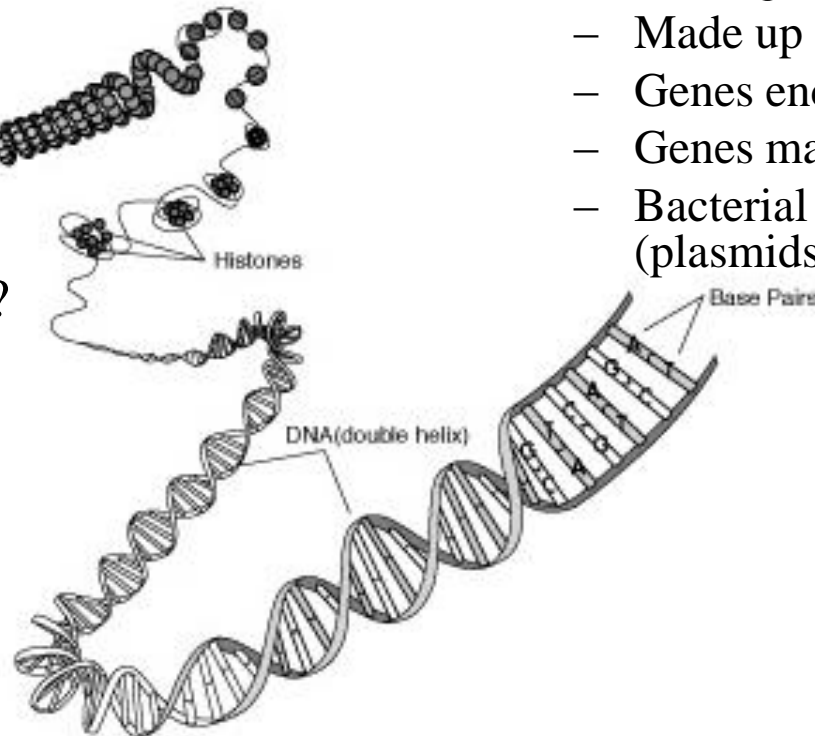
Introduction

- So what is DNA, anyway?
- A long, twisted-ladder molecule
 - Contains four different nucleotide molecules (A, C, T, G).
 - Each base is a letter in the genetic code.



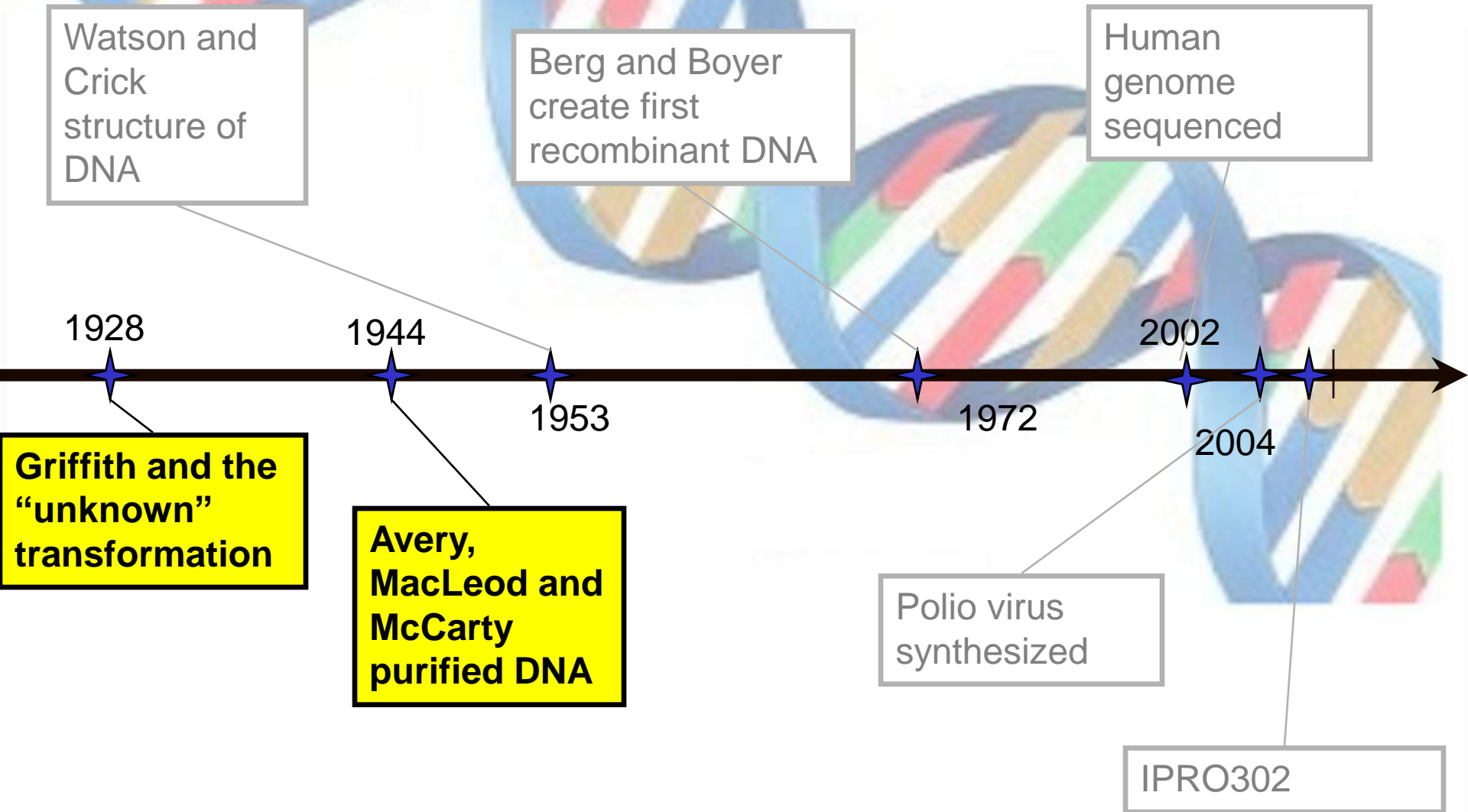
- What are genes?
 - Made up of base pairs
 - Genes encode proteins
 - Genes make up chromosomes
 - Bacterial chromosomes (plasmids) are circular

- What are we doing?
 - Manipulating bacterial genes



Genetics Timeline

Our view of genetics has evolved over time



Watson and Crick structure of DNA

Berg and Boyer create first recombinant DNA

Human genome sequenced

1928

1944

1953

1972

2002

2004

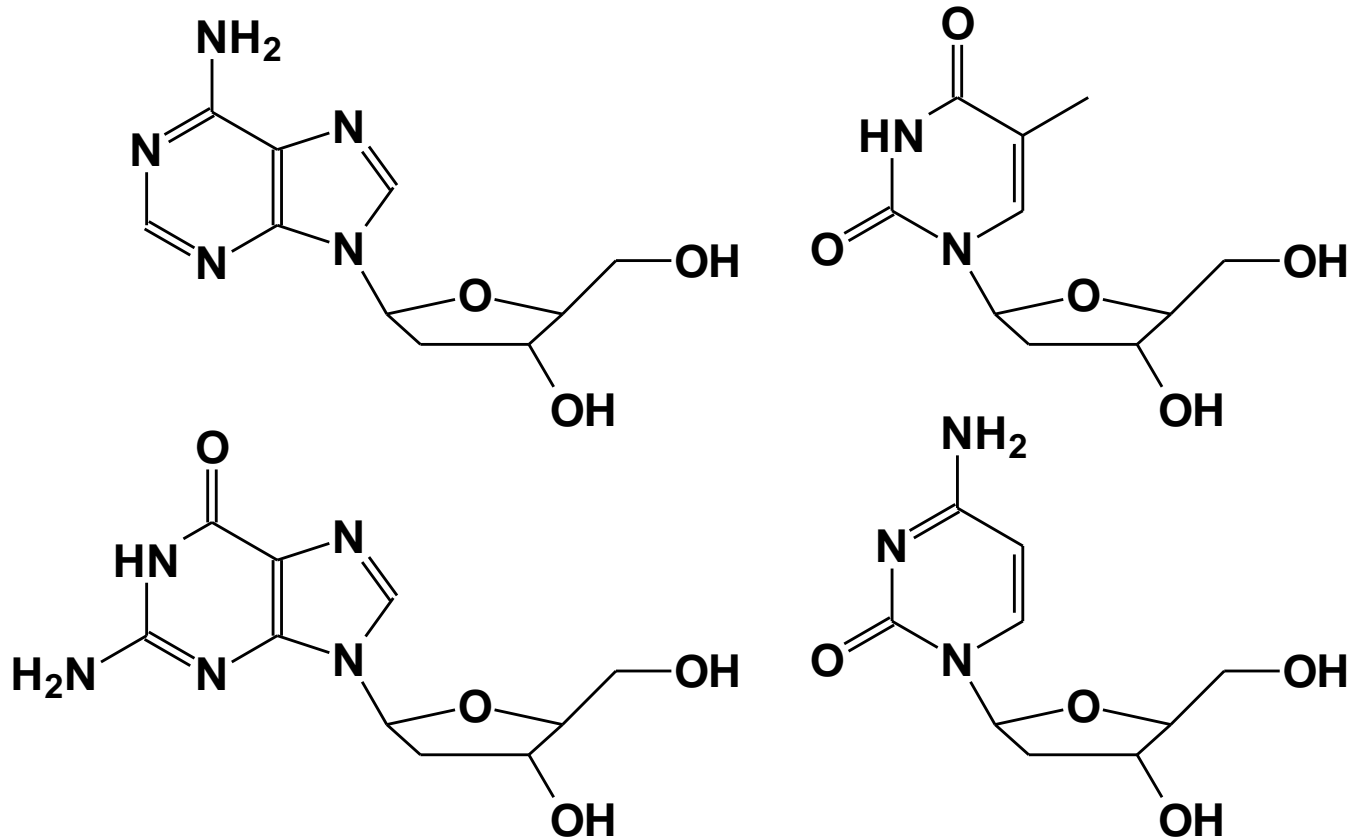
Griffith and the "unknown" transformation

Avery, MacLeod and McCarty purified DNA

Polio virus synthesized

IPRO302

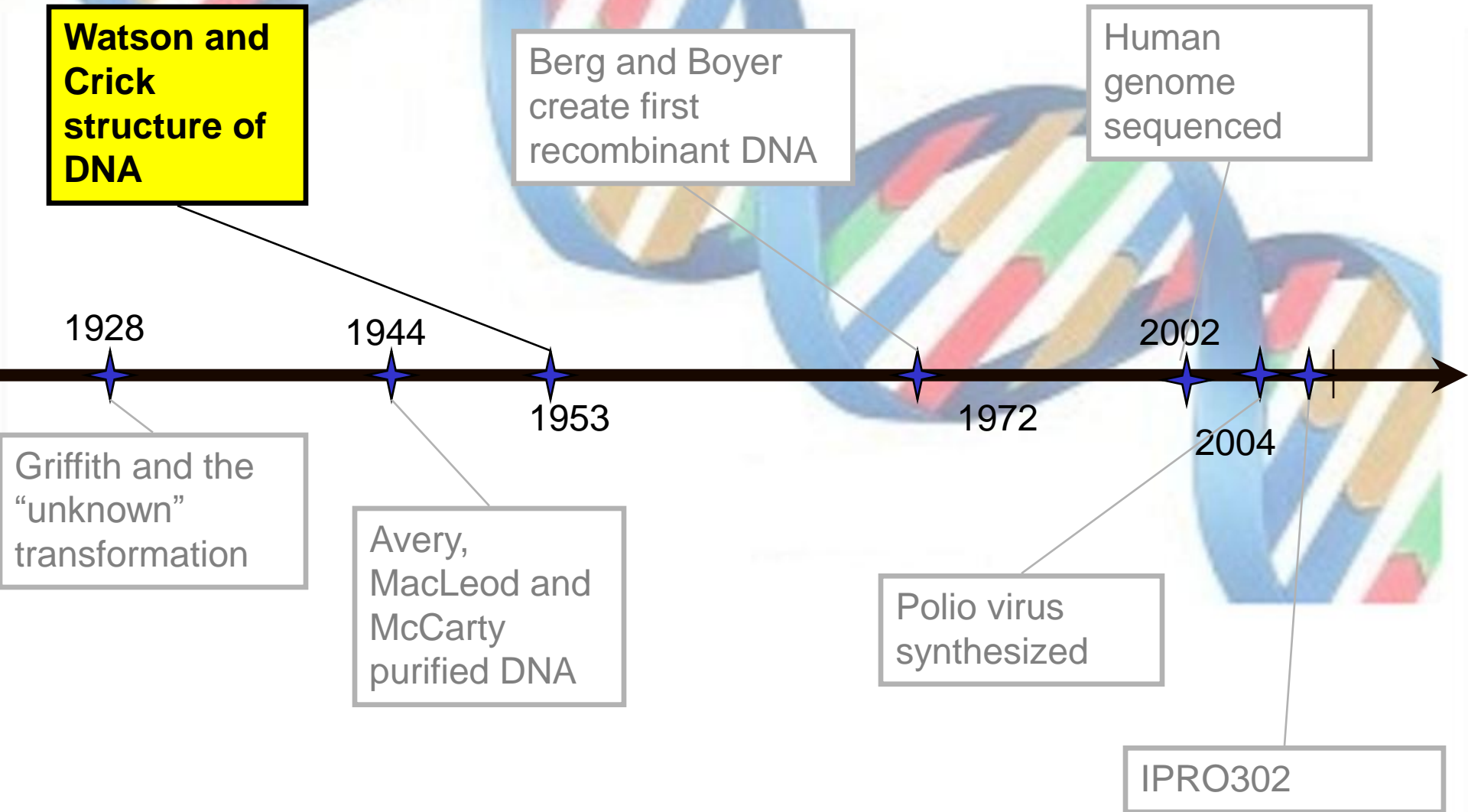
The chemicals of heredity...



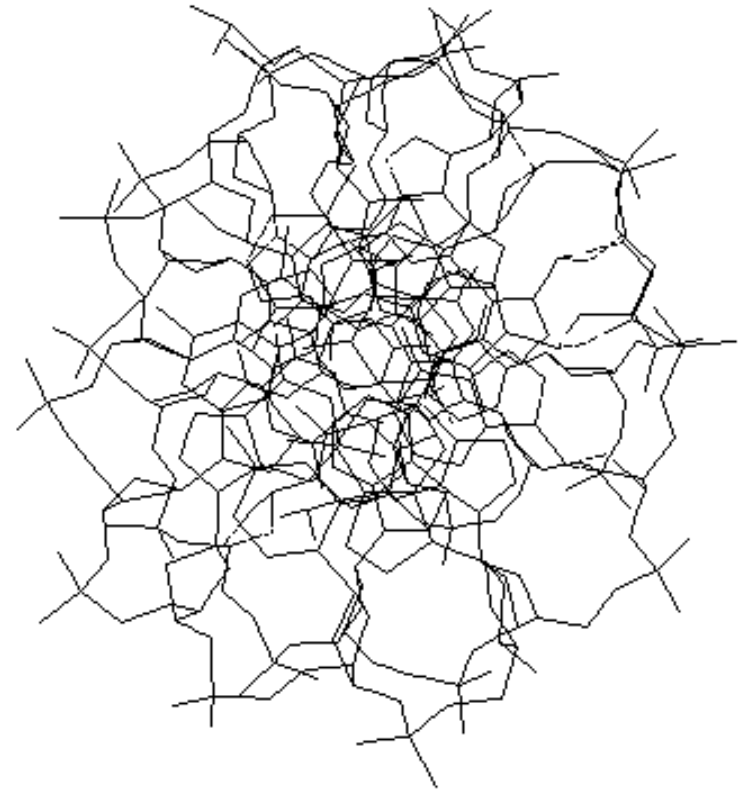
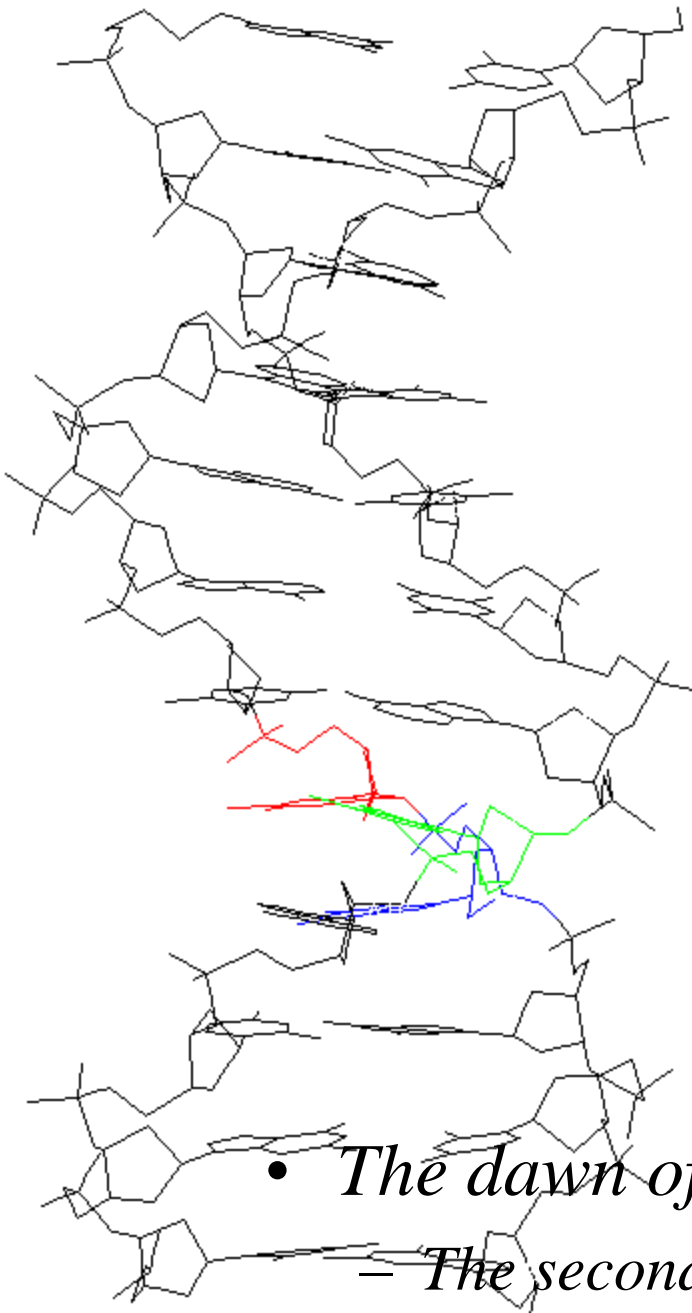
Heredity boils down to chemicals

Genetics Timeline

Our view of genetics has evolved over time



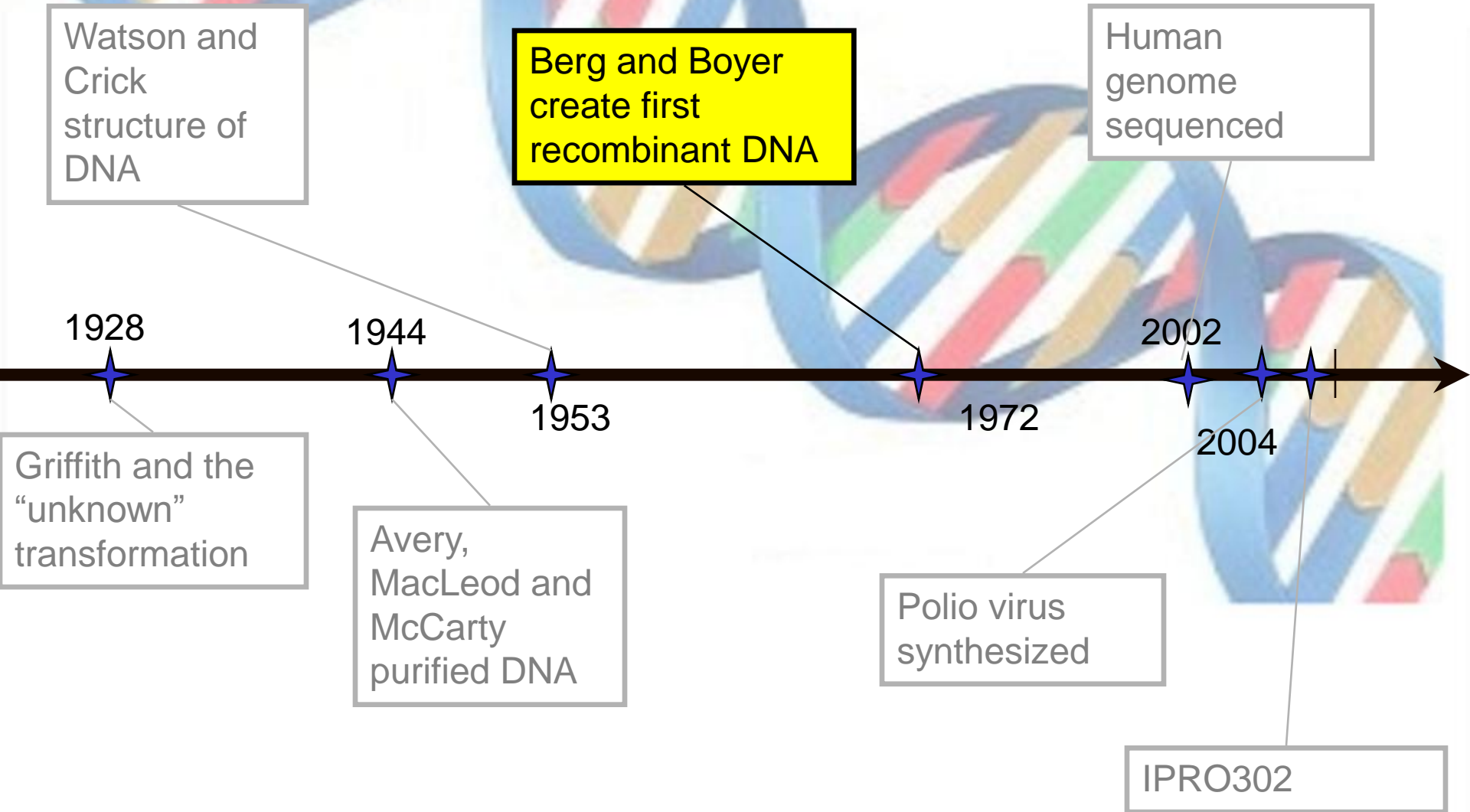
DNA



- *The dawn of the genetic era – the double helix*
= *The second piece of the puzzle falls into place*

Genetics Timeline

Our view of genetics has evolved over time



Speaking the Genetic Language

- We start to see a pattern emerge from the order of chemicals
- This pattern can be said to be its own language
- It tells the cell what messenger RNA to make
- The RNA tells the cell what protein to make
- How do we speak this language?

Letters...

421 CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTCC CATTGCGCCAT TCAGGCTGCG
481 ATCCGCCCCGT CACTAACAC CGTGCCTGTT GACTATTTTA CCTCTGGCGG TGATAATGGT
541 TGCATGTACT AAGGAGGTGA ATTCGTGAAA CCAGTAACGT TATACGATGT CGCAGAGTAT
601 GCCGGTGTCT CTTATCAGAC CGTTTCCCGC GTGGTGAACC AGGCCAGCCA CGTTTCTGCG
661 AAAACGCGGG AAAAAGTGGA AGCGGCGATG GCGGAGCTGA ATTACATTCC CAACCGCGTG
721 GCACAACAAC TGGCGGGCAA ACAGTCGTTG CTGATTGGCG TTGCCACCTC CAGTCTGGCC
781 CTGCACGCGC CGTCGCAAAT TGTCGCGGCG ATTAATCTC GCGCCGATCA ACTGGGTGCC
841 AGCGTGGTGG TGTCGATGGT AGAACGAAGC GCGGTCGAAG CCTGTAAAGC GGCGGTGCAC
901 AATCTTCTCG CGCAACGCGT CAGTGGGCTG ATCATTAACT ATCCGCTGGA TGACCAGGAT
961 GCCATTGCTG TGAAGCTGC CTGCACTAAT GTTCCGGCGT TATTTCTTGA TGTCTCTGAC
1021 CAGACACCCA TCAACAGTAT TATTTTCTCC CATGAAGACG GTACGCGACT GGGCGTGGAG
1081 CATCTGGTCG CATTGGGTCA CCAGCAAATC GCGCTGTTAG CGGGCCCATT AAGTTCTGTC
1141 TCGGCGCGTC TGGCTCTGGC TGGCTGGCAT AAATATCTCA CTCGCAATCA AATTCAGCCG
1201 ATAGCGGAAC GGAAGGCGA CTGGAGTGCC ATGTCCGGTT TTCAACAAAC CATGCAAATG
1261 CTGAATGAGG GCATCGTTCC CACTGCGATG CTGGTTGCCA ACGATCAGAT GCGCTGGGC
1321 GCAATGCGCG CCATTACCGA GTCCGGGCTG CGCGTTGGTG CGGATATCTC GGTAGTGGGA
1381 TACGACGATA CCGAAGACAG CTCATGTTAT ATCCCGCCGT TAACCACCAT CAAACAGGAT
1441 TTTCGCCTGC TGGGGCAAAC CAGCGTGGAC CGCTTGCTGC AACTCTCTCA GGGCCAGGCG
1501 GTGAAGGGCA ATCAGCTGTT GCCCGTCTCA CTGGTGA AAAAACCAC CCTGGCGCCC
1561 AATACGCAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATGCAGCT GGCACGACAG
1621 GTTTCCCGAC TGAAGCGG GCAGGCAGCA AATGATGAGA ATTATGCAGC AGCTGTATAA
1681 GCGGCCGCAA AAAACCCCTC AAGACCCGTT TAGAGGCCCC AAGGGGTTAT GCTACTTAAG
1741 GGGCTAGAGC GGCCCATGTG AGCAAAGGC CAGCAAAGG CCAGGAACCG TAAAAGGCC
1801 GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCTGACG AGCATCACAA AAATCGACGC

It starts with letters

Words...

421 CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTCC CATTCGCCAT TCAGGCTGCG
481 ATCCGCCCGT CGACTAACAC CGTGC GTTT GACTATTTTA CCTCTGGCGG TGATAATGGT
541 TGCATGTACT AAGGAGGTGA ATTCGTGAAA CCAGTAACGT TATACGATGT CGCAGAGTAT
601 GCCGGTGTCT CTTATCAGAC CGTTTCCCGC GTGGTGAACC AGGCCAGCCA CGTTTCTGCG
661 AAAACGCGGG AAAAAGTGGA AGCGGCGATG GCGGAGCTGA ATTACATTCC CAACCGCGTG
721 GCACAACAAC TGGCGGGCAA ACAGTCGTTG CTGATTGGCG TTGCCACCTC CAGTCTGGCC
781 CTGCACGCGC CGTCGCAAAT TGTCGCGGCG ATTAATCTC GCGCCGATCA ACTGGGTGCC
841 AGCGTGGTGG TGTGATGGT AGAACGAAGC GCGGTCGAAG CCTGTAAAGC GGCGGTGCAC
901 AATCTTCTCG CGCAACGCGT CAGTGGGCTG ATCATTAACT ATCCGCTGGA TGACCAGGAT
961 GCCATTGCTG TGGAAAGCTGC CTGCACTAAT GTTCCGGCGT TATTTCTTGA TGTCTCTGAC
1021 CAGACACCCA TCAACAGTAT TATTTTCTCC CATGAAGACG GTACGCGACT GGGCGTGGAG
1081 CATCTGGTCG CATTGGGTCA CCAGCAAATC GCGCTGTTAG CGGGCCCAT T AAGTTCTGTC
1141 TCGGCGCGTC TGGCTCTGGC TGGCTGGCAT AAATATCTCA CTCGCAATCA AATTCAGCCG
1201 ATAGCGGAAC GGAAGGCGA CTGGAGTGCC ATGTCCGGTT TTCAACAAC CATGCAAATG
1261 CTGAATGAGG GCATCGTTCC CACTGCGATG CTGGTTGCCA ACGATCAGAT GGCGCTGGGC
1321 GCAATGCGCG CCATTACCGA GTCCGGGCTG CGCGTTGGTG CGGATATCTC GGTAGTGGGA
1381 TACGACGATA CCGAAGACAG CTCATGTTAT ATCCCGCCGT TAACCACCAT CAAACAGGAT
1441 TTTCGCCTGC TGGGGCAAAC CAGCGTGGAC CGCTTGCTGC AACTCTCTCA GGGCCAGGCG
1501 GTGAAGGGCA ATCAGCTGTT GCCCGTCTCA CTGGTGAAAA GAAAAACCAC CCTGGCGCCC
1561 AATACGCAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATGCAGCT GGCACGACAG
1621 GTTTCCCGAC TGGAAAGCGG GCAGGCAGCA AATGATGAGA ATTATGCAGC AGCTGTATAA
1681 GCGGC CGCAA AAAACCCCTC AAGACCCGTT TAGAGGCCCC AAGGGGTTAT GCTACTTAAG
1741 GGGCTAGAGC GGCCCATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAGGCC
1801 GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC

Then we progress to words

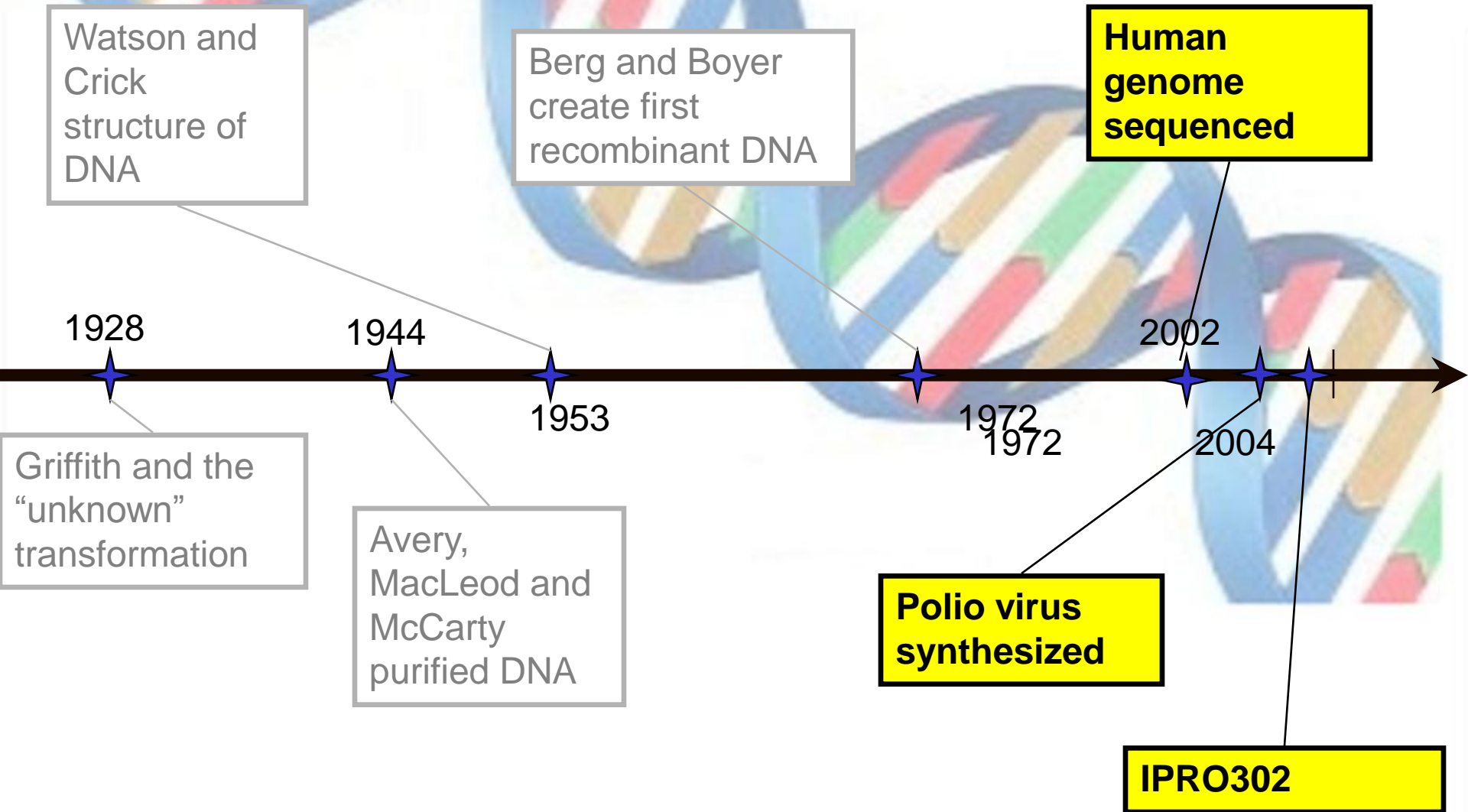
Meanings



And then we go to paragraphs...

Genetics Timeline

Our view of genetics has evolved over time

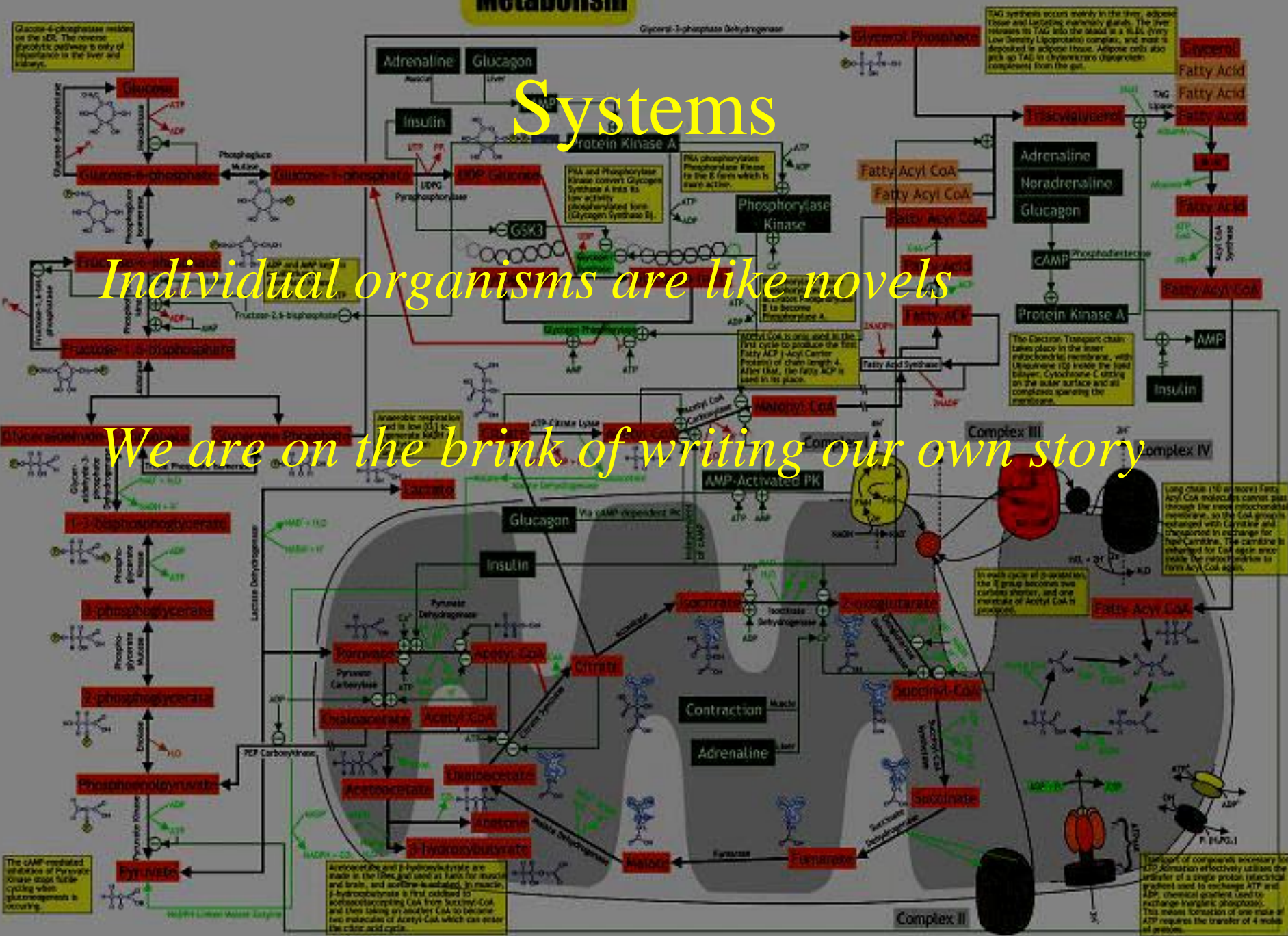


Metabolism

Systems

Individual organisms are like novels

We are on the brink of writing our own story



The cAMP-mediated inhibition of Pyruvate Kinase stops futile cycling when gluconeogenesis is occurring.

Acetyl CoA and β -hydroxybutyrate are made in the liver and used as fuels for muscle and brain, and acetyl-CoA is used in muscle. β -hydroxybutyrate is first oxidized to acetoacetyl-CoA from succinyl-CoA and then taking in another CoA to become two molecules of Acetyl CoA which can enter the citric acid cycle.

TAG synthesis occurs mainly in the liver, adipose tissue and lactating mammary glands. The liver releases TAG into the blood as a HDL (Very Low Density Lipoprotein) complex, and most is deposited in adipose tissue. Adipose cells also pick up TAG in chylomicron lipoprotein complexes from the gut.

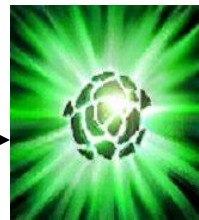
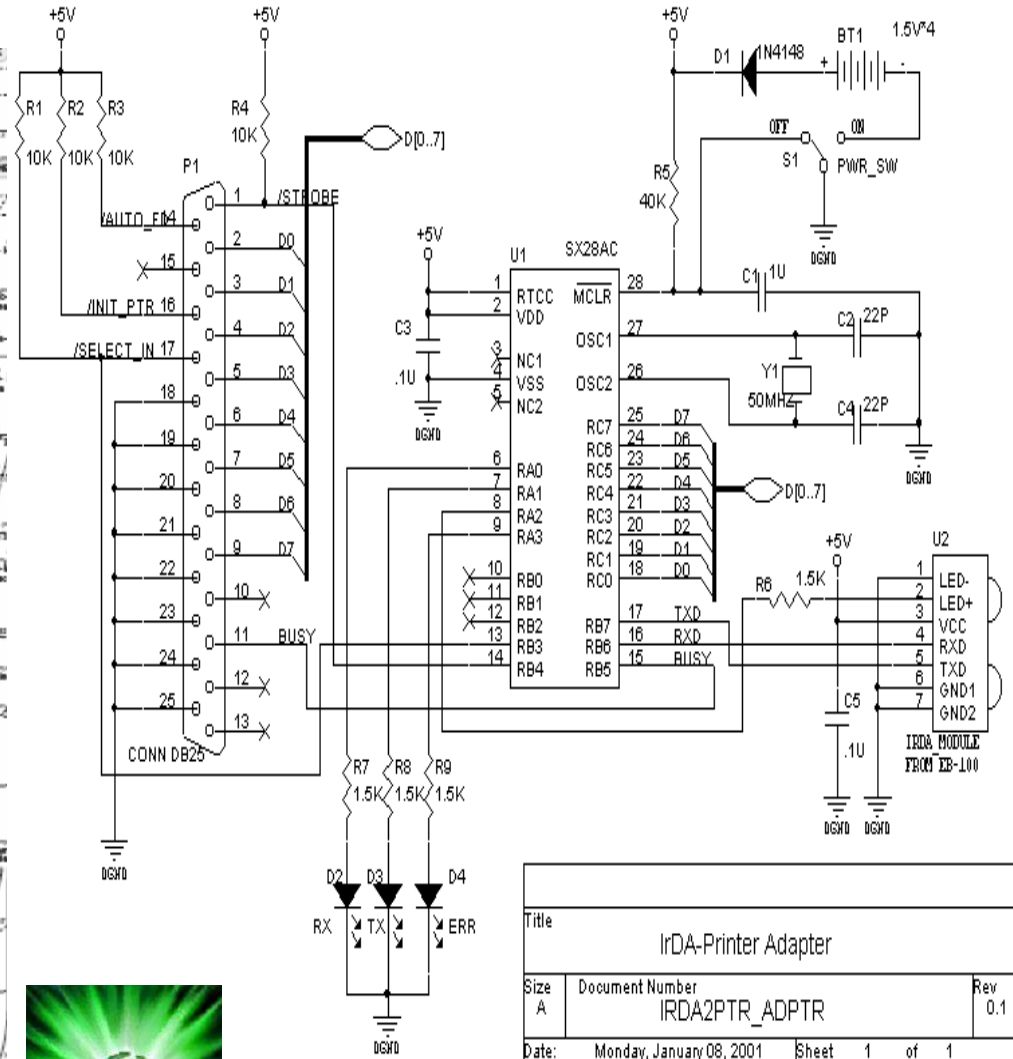
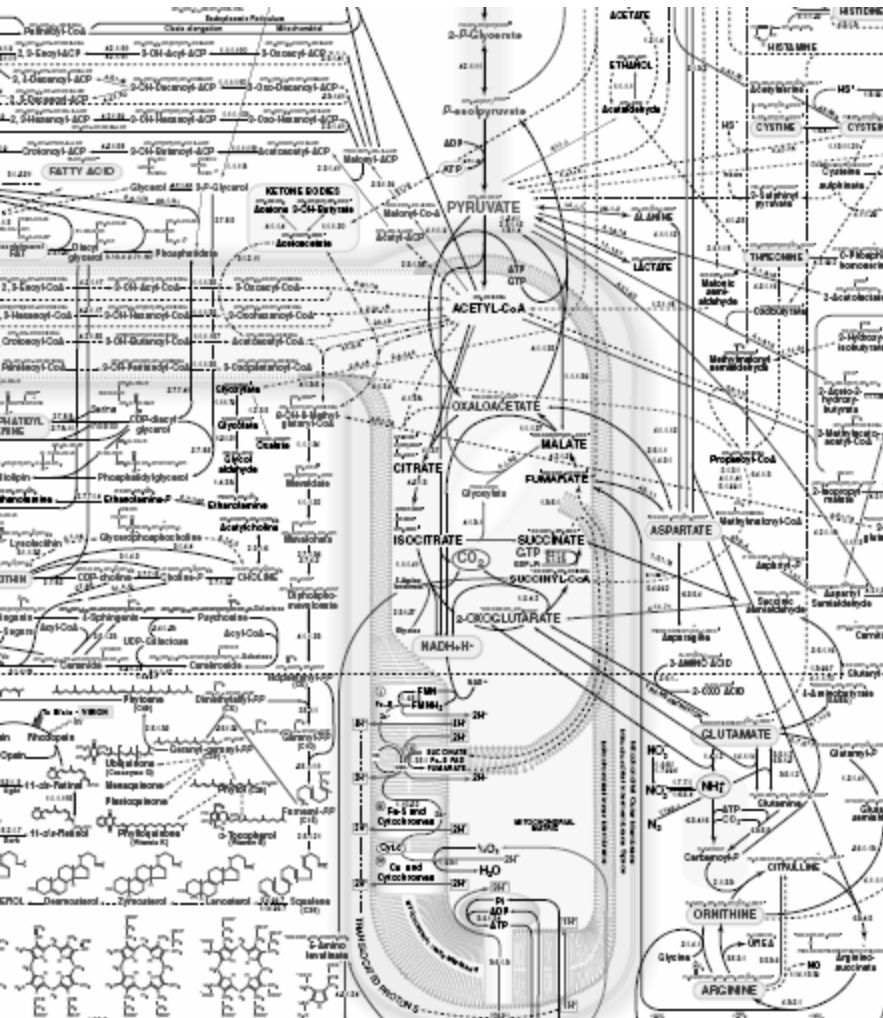
The Electron Transport chain takes place in the inner mitochondrial membrane, with Ubiquinone (Q) in the lipid bilayer. Cytochrome C sitting on the outer surface and all complexes spanning the membrane.

Long chain (16 or more) Fatty Acyl CoA molecules cannot pass through the inner mitochondrial membrane, so the CoA groups are exchanged with Carnitine and transported in exchange for Fatty Carnitine. The carnitine is exchanged for CoA again inside the matrix to form Fatty Acyl CoA again.

In each cycle of β -oxidation, the β group becomes two carbons shorter, and one molecule of Acetyl CoA is produced.

Large part of complexes necessary for ATP formation effectively utilizes the steeper of a single proton (electrical gradient) used to exchange ATP and ADP. Chemical gradient used to exchange hydrogen phosphate. This means formation of one mole of ATP requires the transfer of 4 moles of protons.

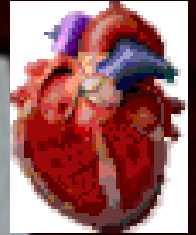
Biology and Engineering Collide



Metabolic Pathways

Circuits Schematic

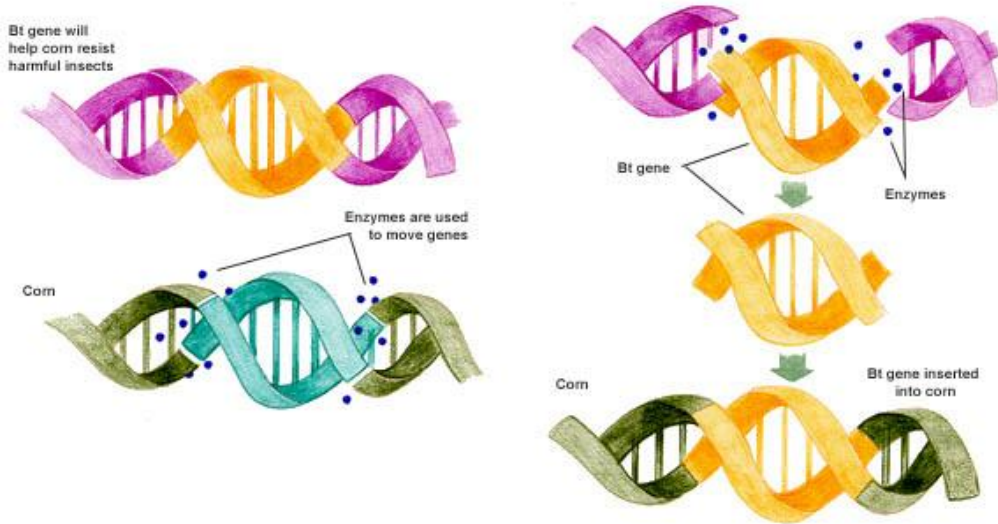
Our test case



- **If not a novel, then at least a pamphlet**
- **A simple genetic circuit**
 - Emergent property that cannot be linked to a single component
 - Small enough to build with current technology and resources
- **An Oscillator**
 - Linked genes - bounce between ON / OFF
 - fluorescent proteins to visualize/output
- **So, why should you care?**
 - Many biological oscillators
 - heartbeats to breathing, functions in an oscillatory system
 - A beginning to more complicated circuits
 - DNA computers? Artificial intelligence using life itself?

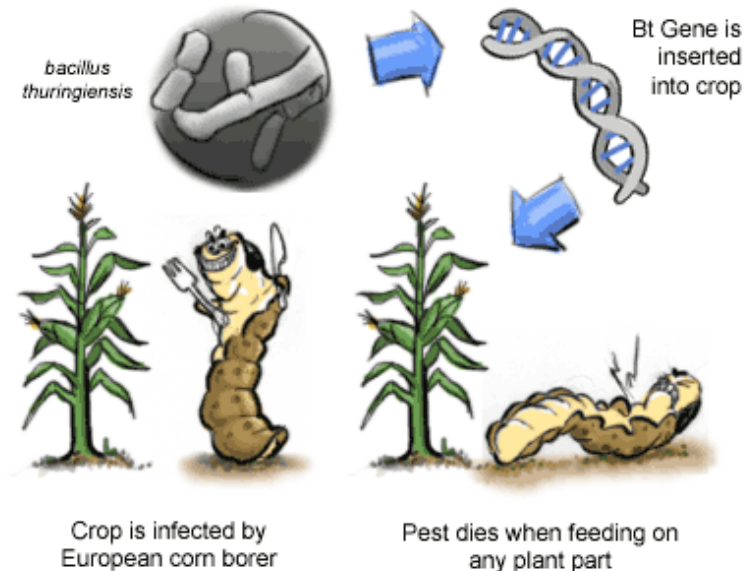


Our Project Goal

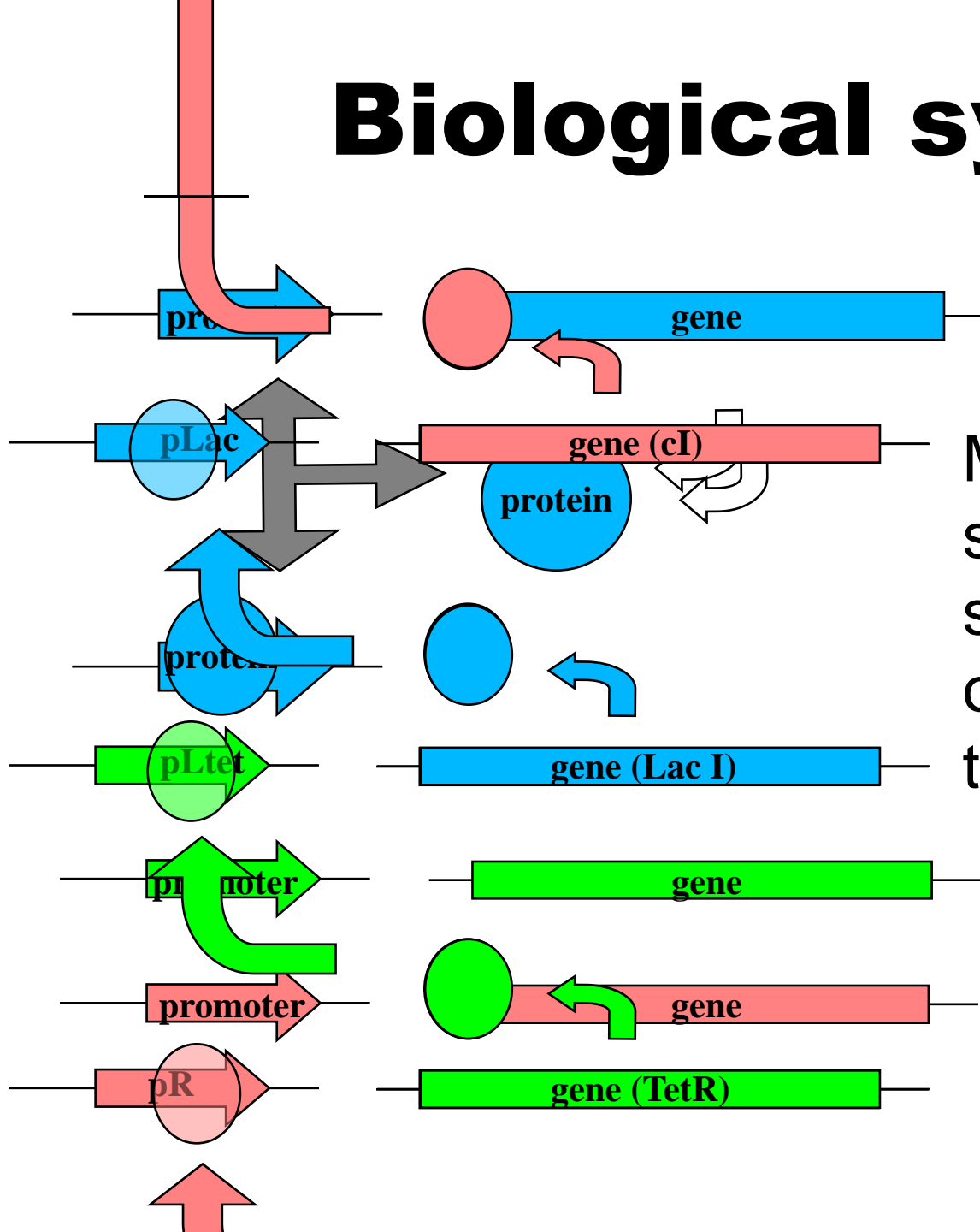


- Design a genetic oscillating system by modifying and combining existing gene systems

- Inserting a single feature in an organism has become common practice
- Our projects goal is to combine features together and create a novel behavior



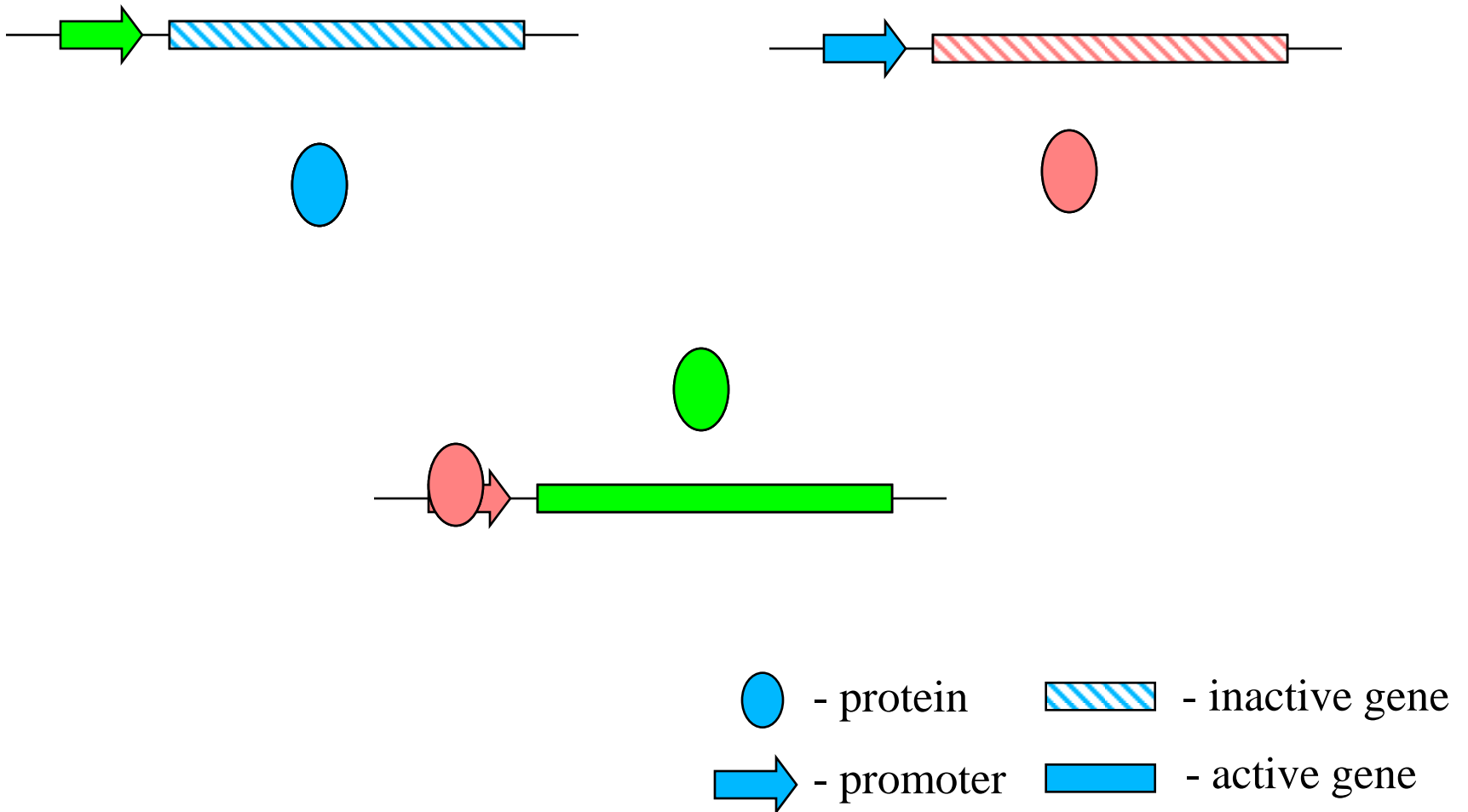
Biological system



Many natural feedback systems come in pairs such that the product of each gene inhibits the gene itself

From Biology to Engineering

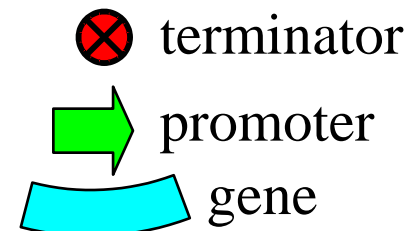
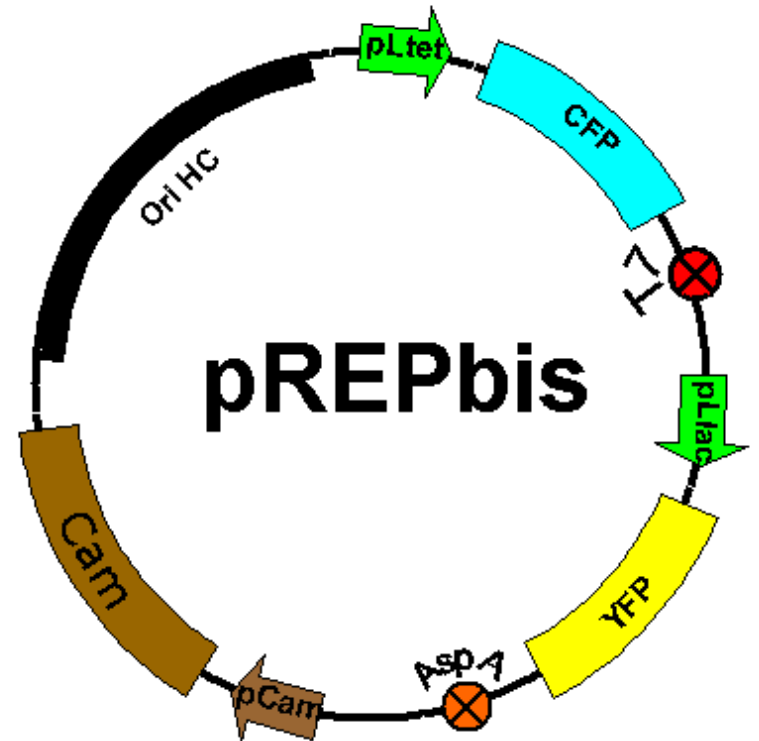
Rearrange gene pieces to obtain an oscillating system – how it all works together?



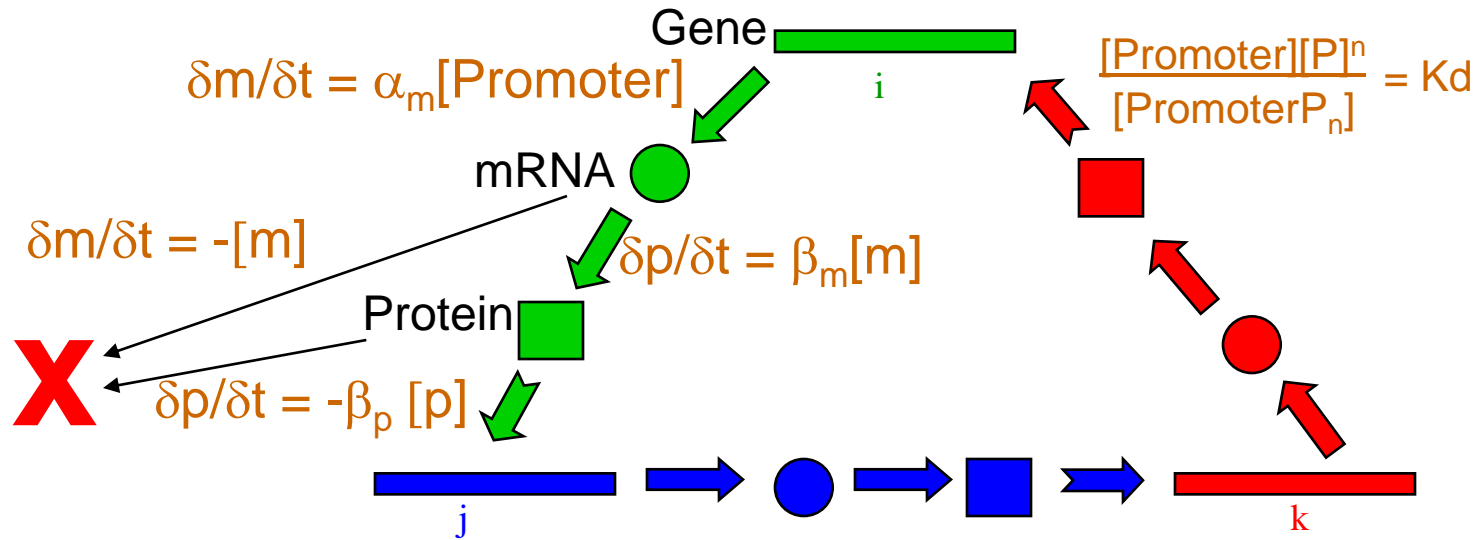
Reporter plasmid

Cannot see the oscillations!!!

- add fluorescent proteins and make the bacteria change color as the system oscillates
- duplicate promoter to track active genes



Modeling



mRNA Equations:

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{1 + p_k^n}$$

$$\frac{dm_j}{dt} = -m_j + \frac{\alpha}{1 + p_i^n}$$

$$\frac{dm_k}{dt} = -m_k + \frac{\alpha}{1 + p_j^n}$$

Protein Equations:

$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

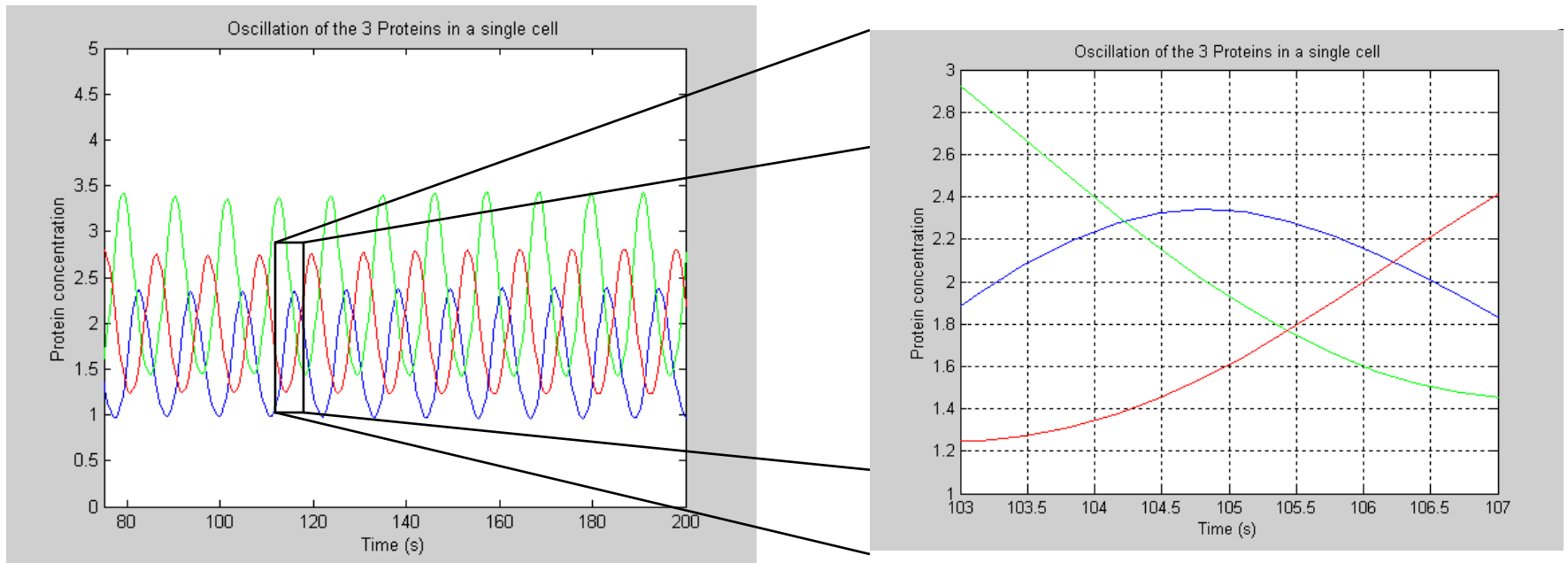
$$\frac{dp_j}{dt} = -\beta(p_j - m_j)$$

$$\frac{dp_k}{dt} = -\beta(p_k - m_k)$$

Matlab

Promoter	replication origin of Vector	copy number in log Phase	Promoter strength (RLU/cell x 10 ⁻⁴)		Regulatory range
			-aTc	+aTc	
PLtetO-1	ColE1	50 - 70	11	27900	2535
	p15A	20 - 30	3,5	12850	3670
	pSC101*	3 - 4	0,4	2020	5050
PLlacO-1	ColE1	50 - 70	-IPTG	+IPTG	620
PA1lacO-1	ColE1	50 - 70	35	21630	350
			30	10430	350

Source: Lutz, R. & Bujard, H. (1997) *Nucleic Acids Research* 25, 1205.





Progress



Fall 2004:

Genes were located and ordered

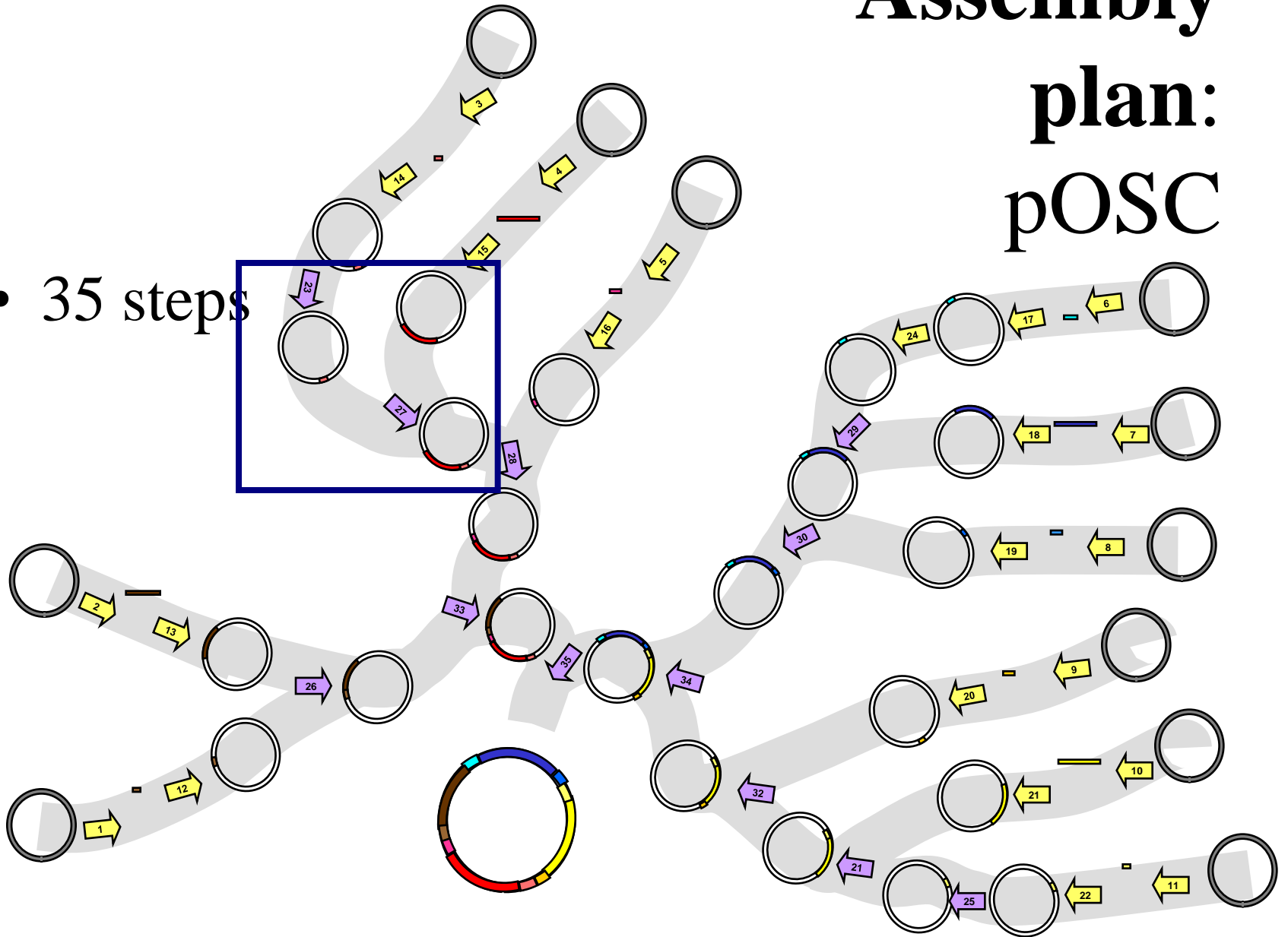
Mathematical modeling was preformed

Spring 2005:

Gene Bank was created

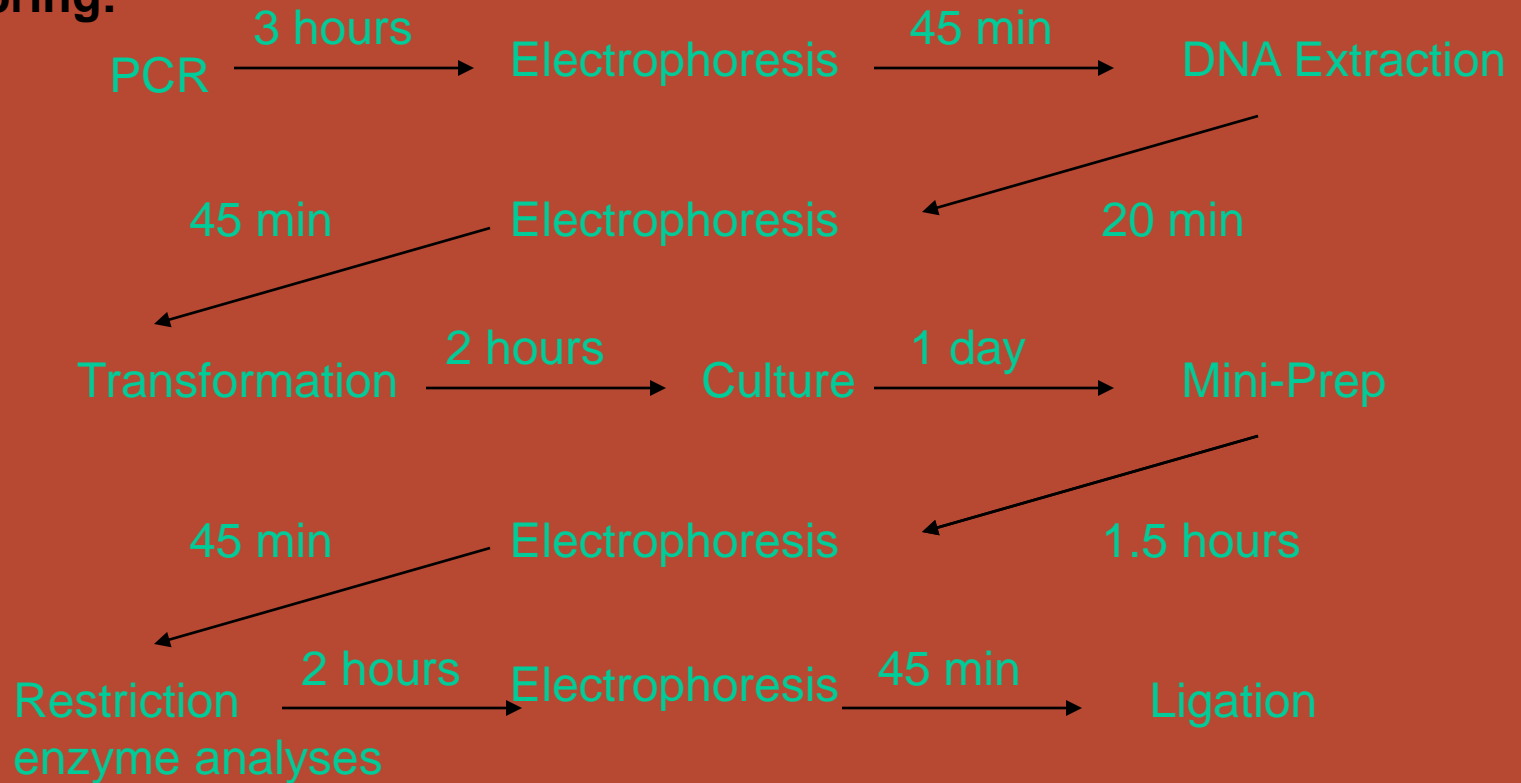
Assembly plan: pOSC

- 35 steps

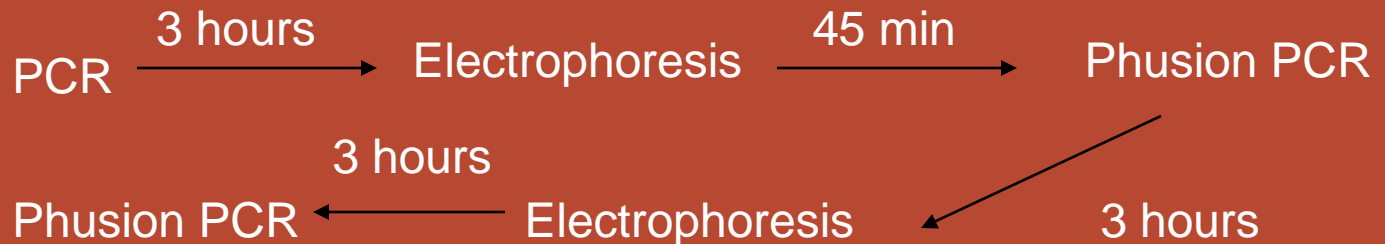


Fall 2005: Project revisions

Spring:

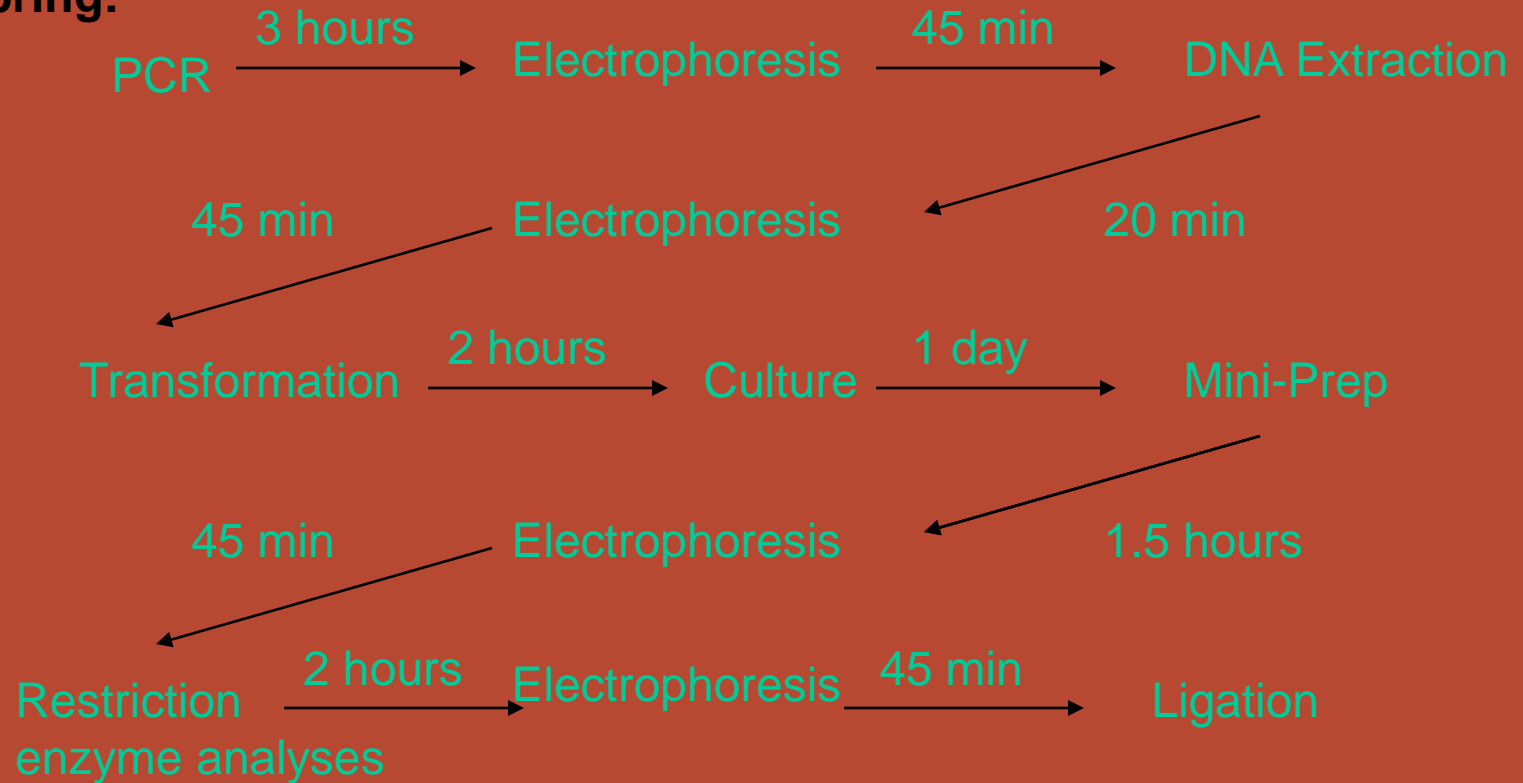


Fall:

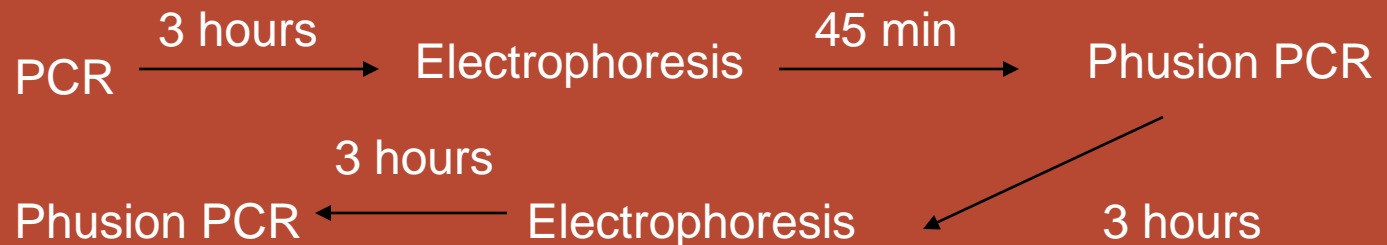


Fall 2005: Project revisions

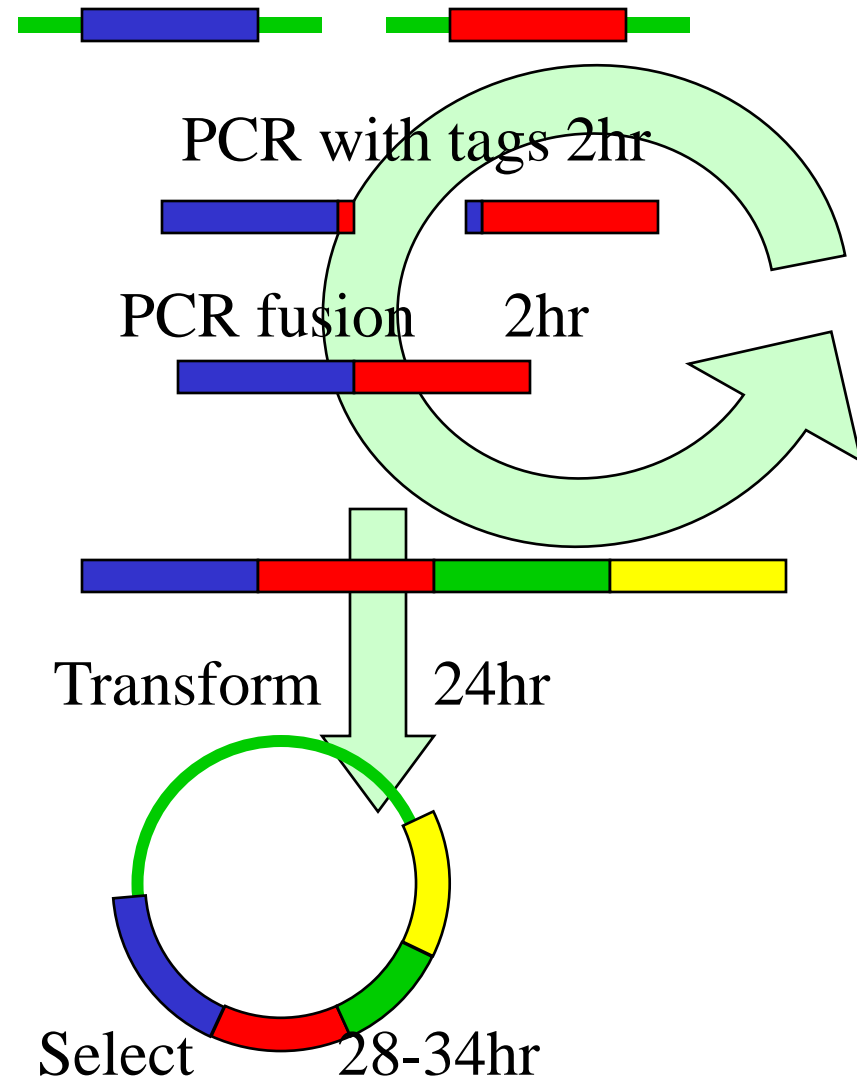
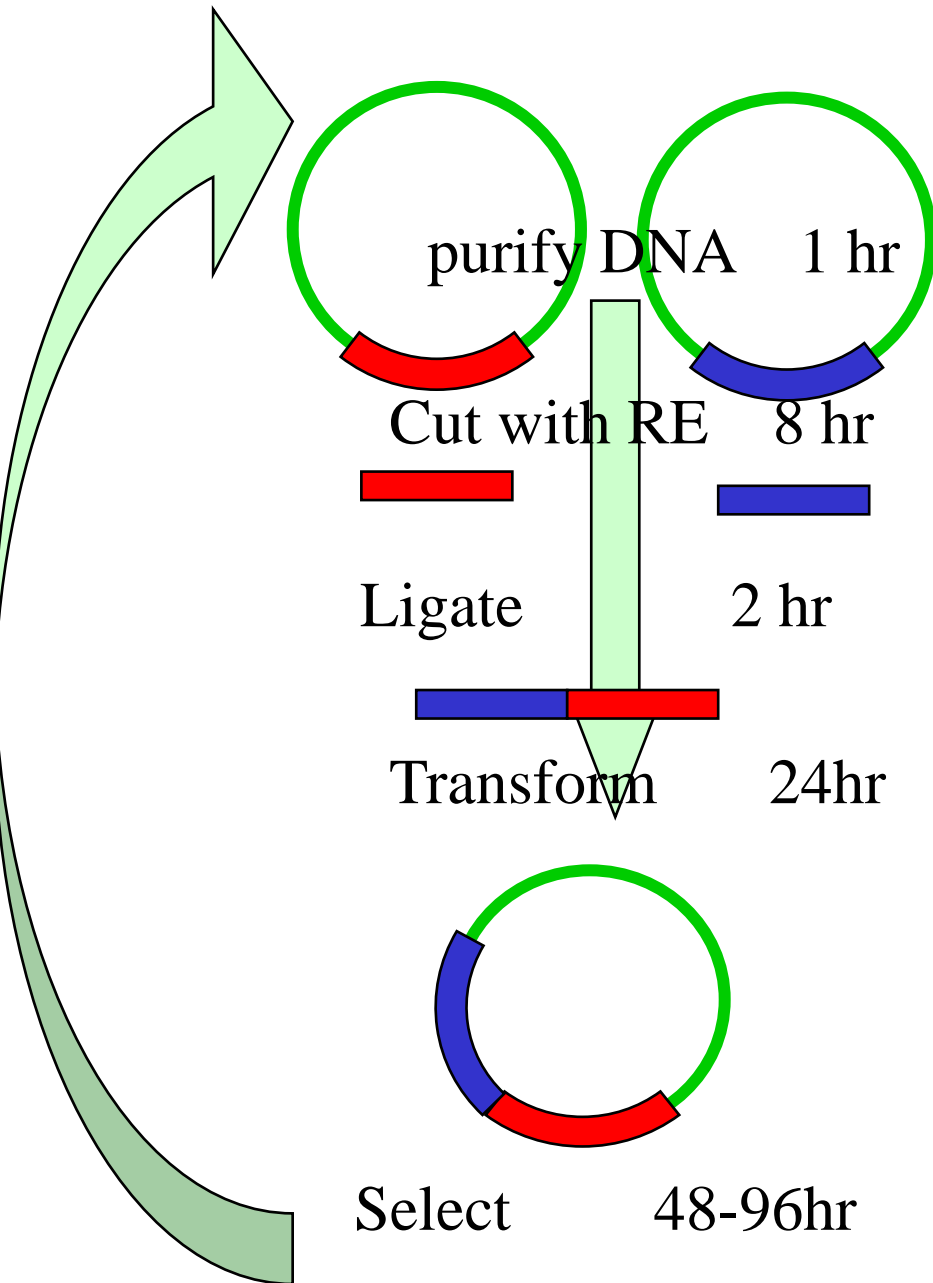
Spring:



Fall:

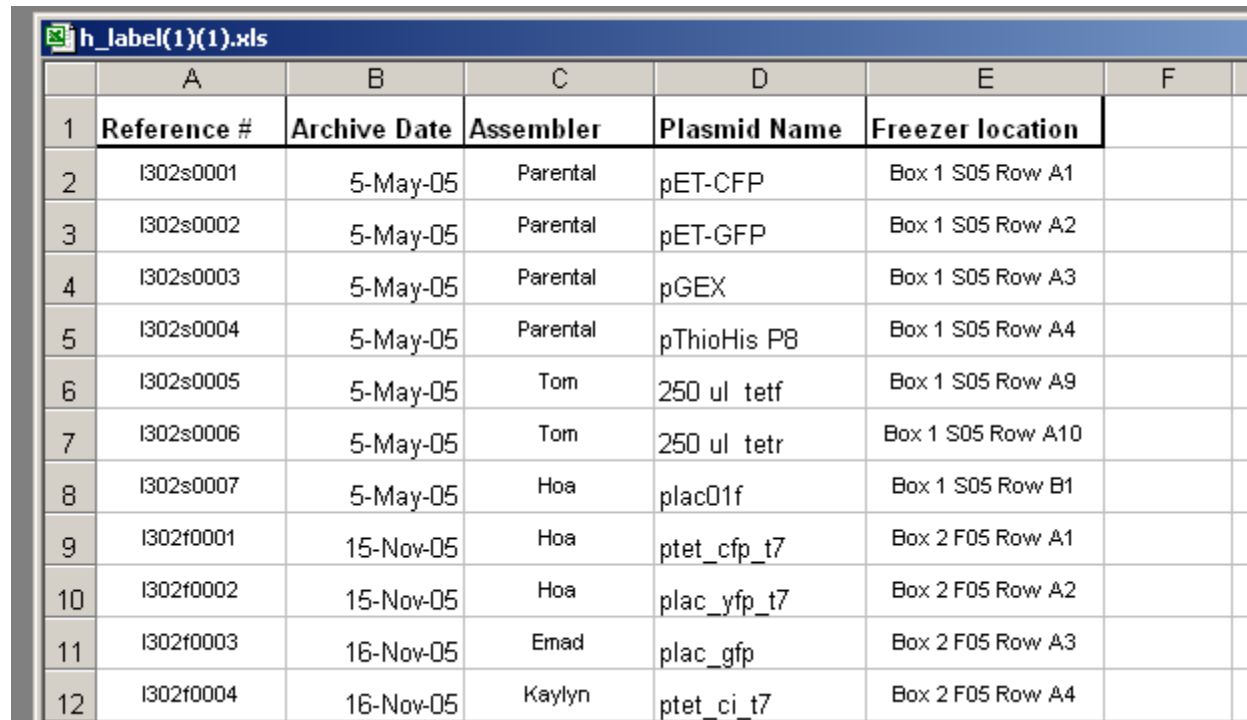


Protocol revision



Results

- All modules for the oscillator system and monitor systems were assembled
- Modules were archived in the Gene Bank



	A	B	C	D	E	F
1	Reference #	Archive Date	Assembler	Plasmid Name	Freezer location	
2	I302s0001	5-May-05	Parental	pET-CFP	Box 1 S05 Row A1	
3	I302s0002	5-May-05	Parental	pET-GFP	Box 1 S05 Row A2	
4	I302s0003	5-May-05	Parental	pGEX	Box 1 S05 Row A3	
5	I302s0004	5-May-05	Parental	pThioHis P8	Box 1 S05 Row A4	
6	I302s0005	5-May-05	Tom	250 ul tetf	Box 1 S05 Row A9	
7	I302s0006	5-May-05	Tom	250 ul tetr	Box 1 S05 Row A10	
8	I302s0007	5-May-05	Hoa	plac01f	Box 1 S05 Row B1	
9	I302f0001	15-Nov-05	Hoa	ptet_cfp_t7	Box 2 F05 Row A1	
10	I302f0002	15-Nov-05	Hoa	plac_yfp_t7	Box 2 F05 Row A2	
11	I302f0003	16-Nov-05	Emad	plac_gfp	Box 2 F05 Row A3	
12	I302f0004	16-Nov-05	Kaylyn	ptet_ci_t7	Box 2 F05 Row A4	

Three monitoring systems
were assembled

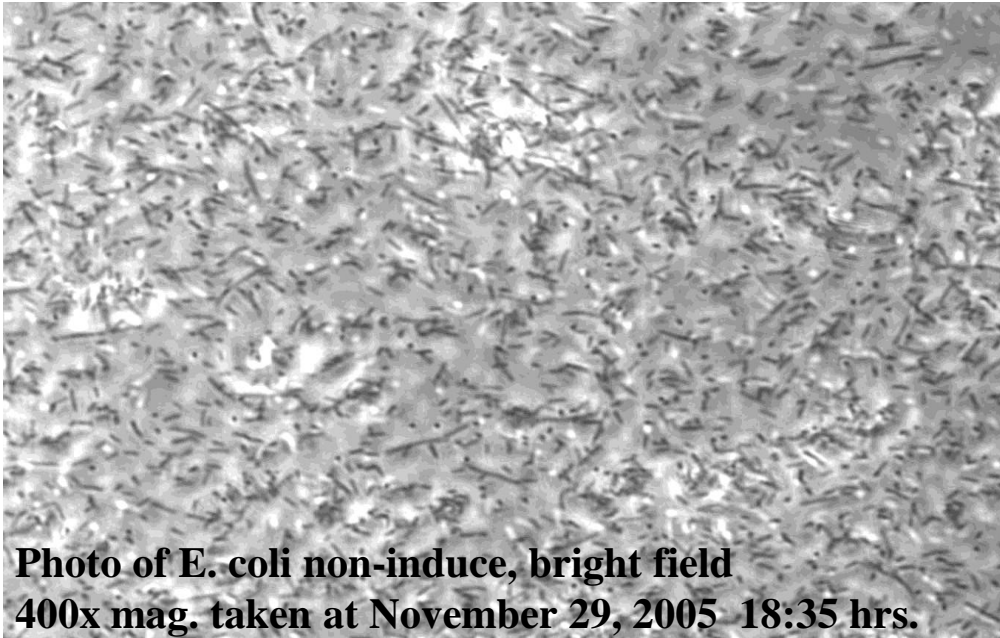


Photo of *E. coli* non-induce, bright field
400x mag. taken at November 29, 2005 18:35 hrs.

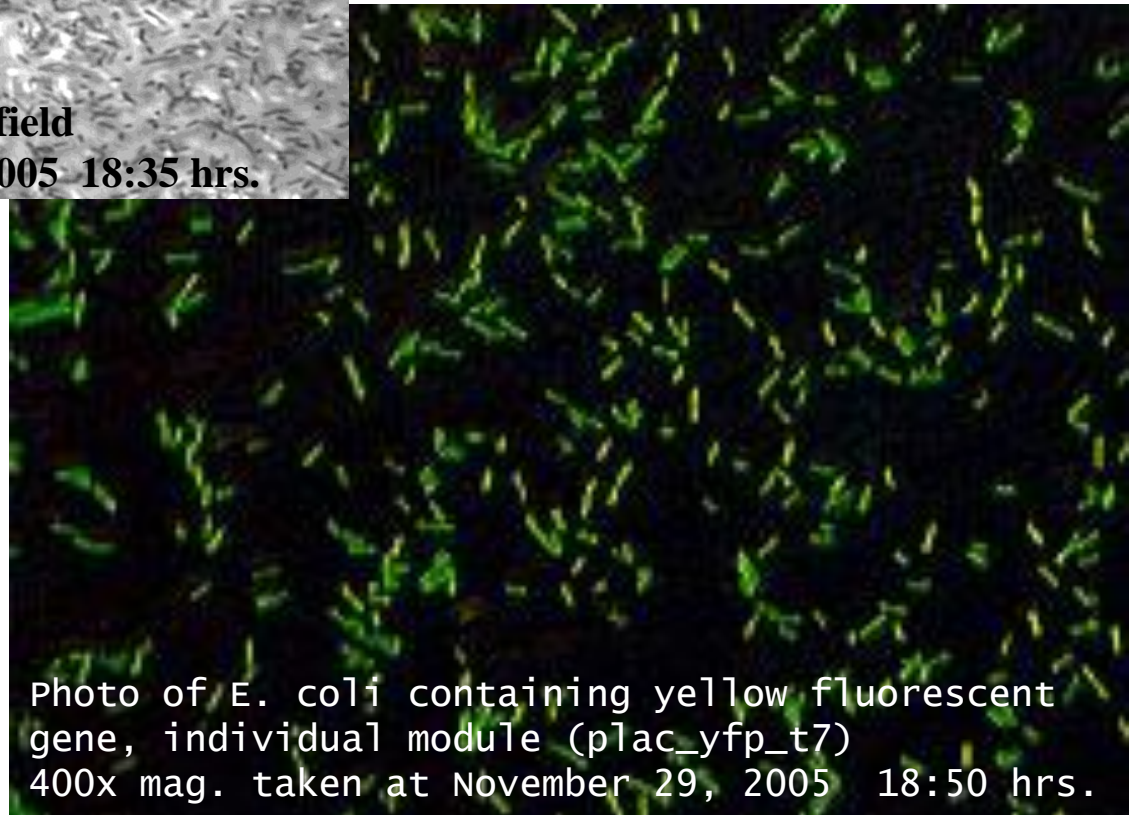


Photo of *E. coli* containing yellow fluorescent
gene, individual module (p_{lac}_yfp_t7)
400x mag. taken at November 29, 2005 18:50 hrs.

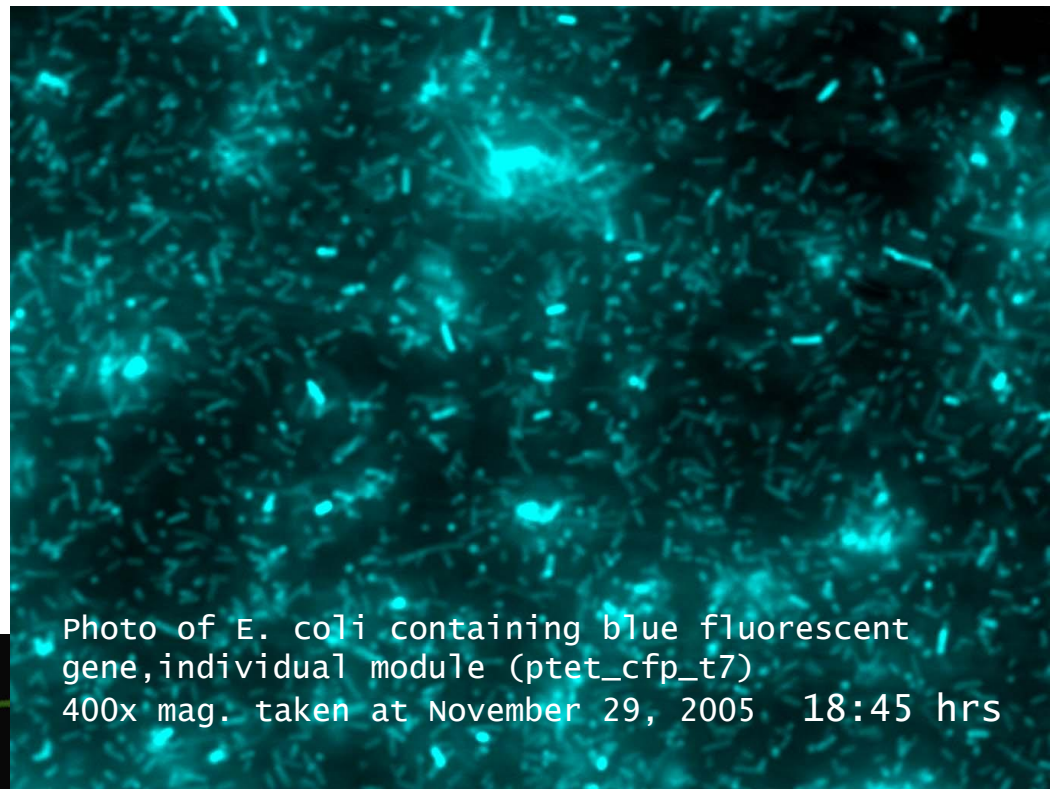


Photo of E. coli containing blue fluorescent
gene, individual module (ptet_cfp_t7)
400x mag. taken at November 29, 2005 18:45 hrs

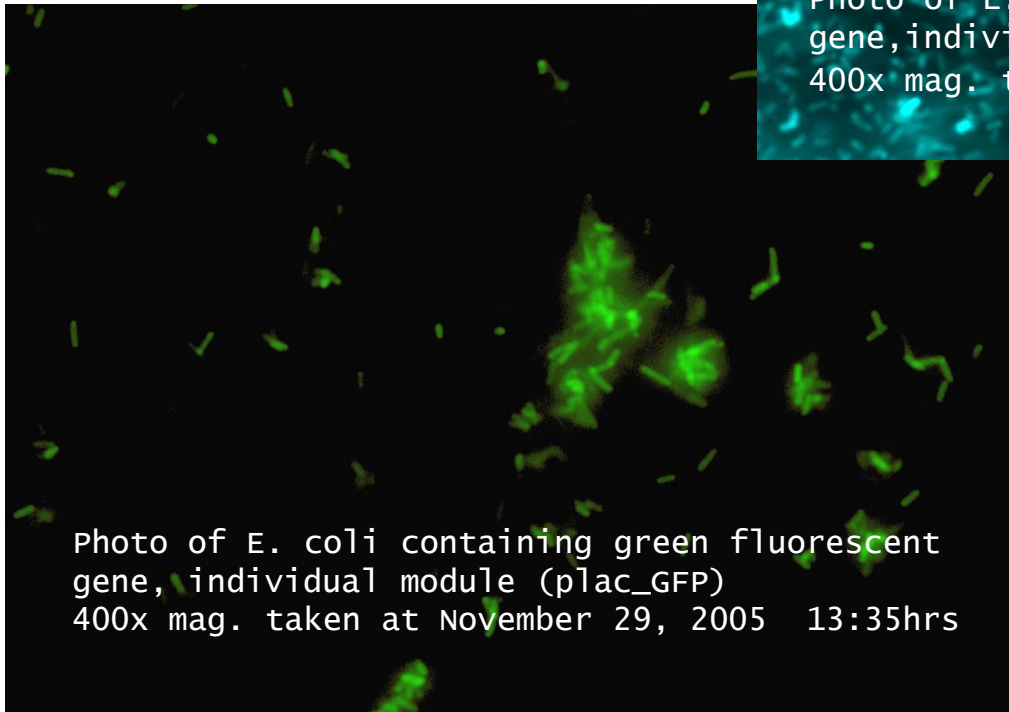
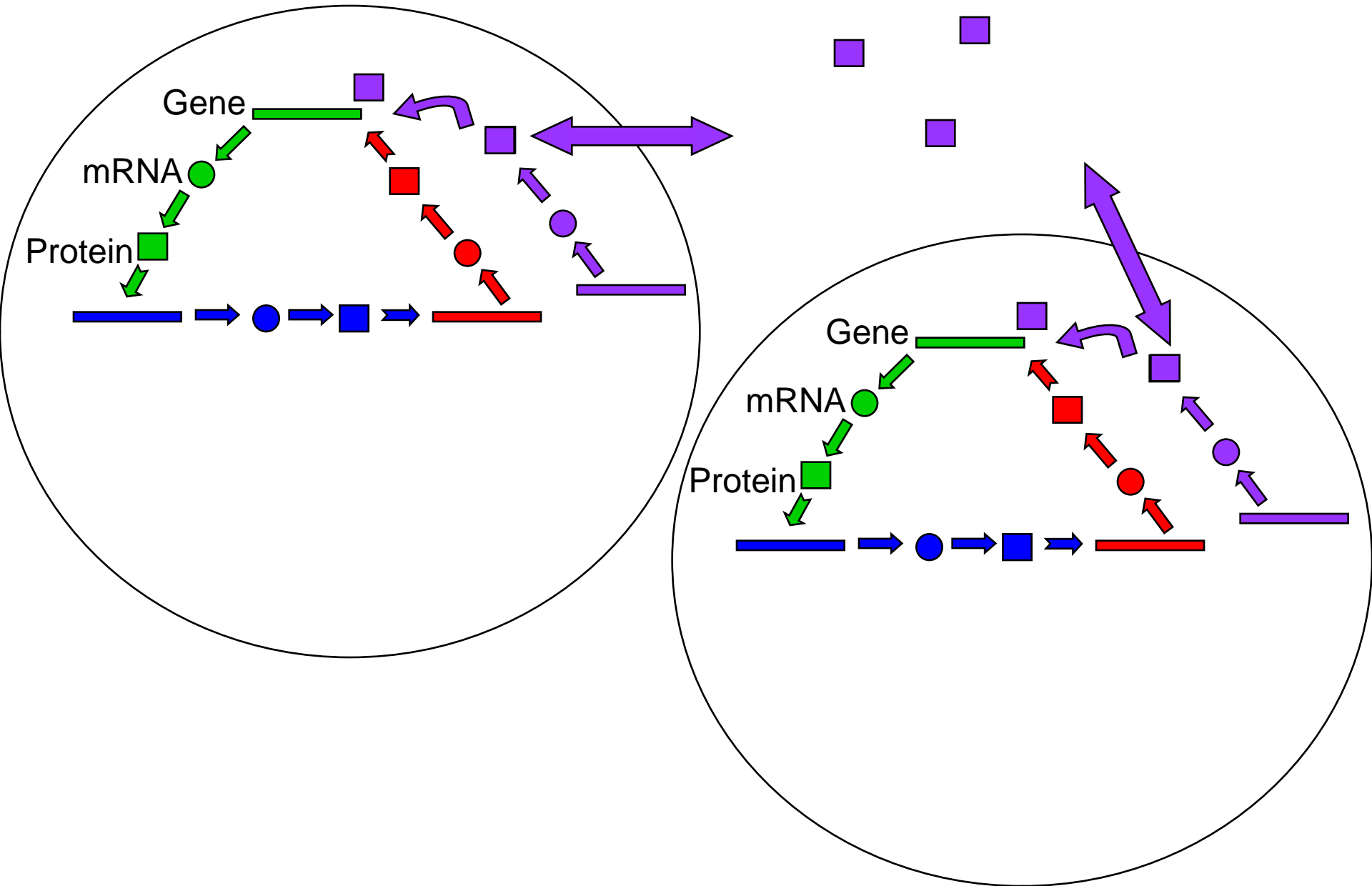


Photo of E. coli containing green fluorescent
gene, individual module (plac_GFP)
400x mag. taken at November 29, 2005 13:35hrs

Synchronization



Modeling

mRNA Equations:

$$\frac{dm_1}{dt} = -m_1 + \frac{\alpha}{1 + p_k^n} + \frac{kS_{int}}{1 + S_{int}}$$

$$\frac{dm_j}{dt} = -m_j + \frac{\alpha}{1 + p_i^n}$$

$$\frac{dm_k}{dt} = -m_k + \frac{\alpha}{1 + p_j^n}$$

Protein Equations:

$$\frac{dp_1}{dt} = -\beta(p_1 - m_1)$$

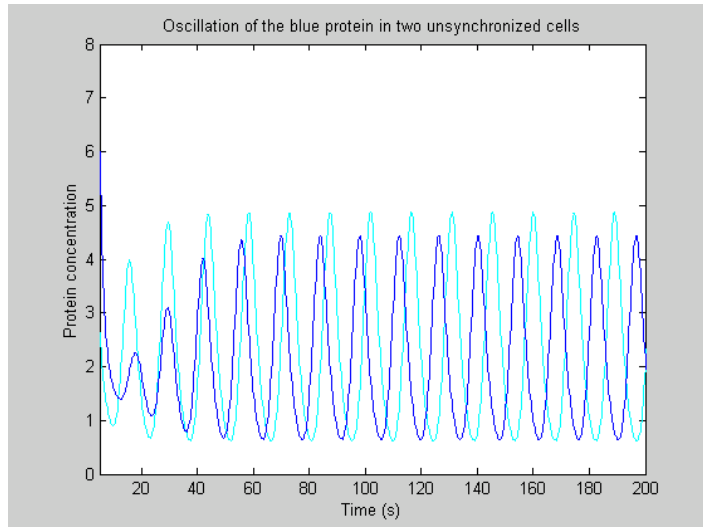
$$\frac{dp_j}{dt} = -\beta(p_j - m_j)$$

$$\frac{dp_k}{dt} = -\beta(p_k - m_k)$$

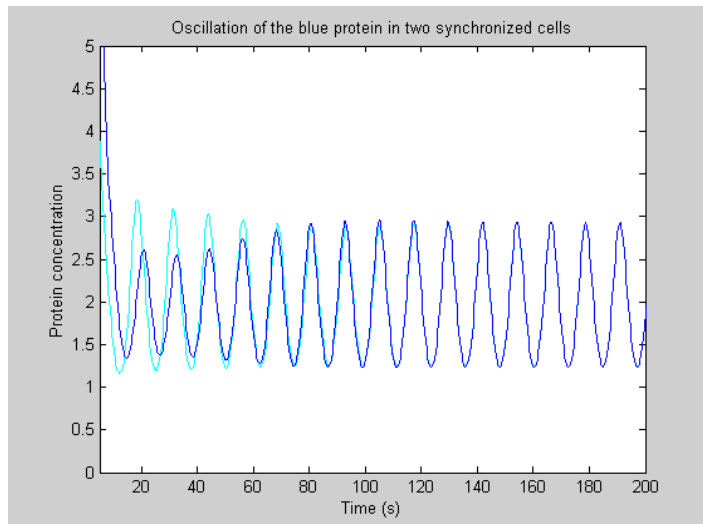
$$\frac{dS_{int}}{dt} = -k_{s0} S_{int} + k_{s1} p_k - \eta(S_{int} - S_{ext})$$

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

Matlab

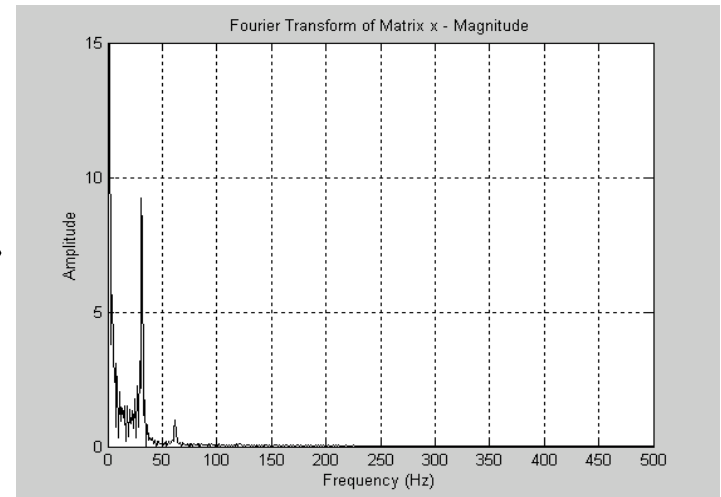


Unsynchronized



Synchronized !

Fourier
Transform



Future Work



Danio rerio

Application to vertebrates

Thank you!

