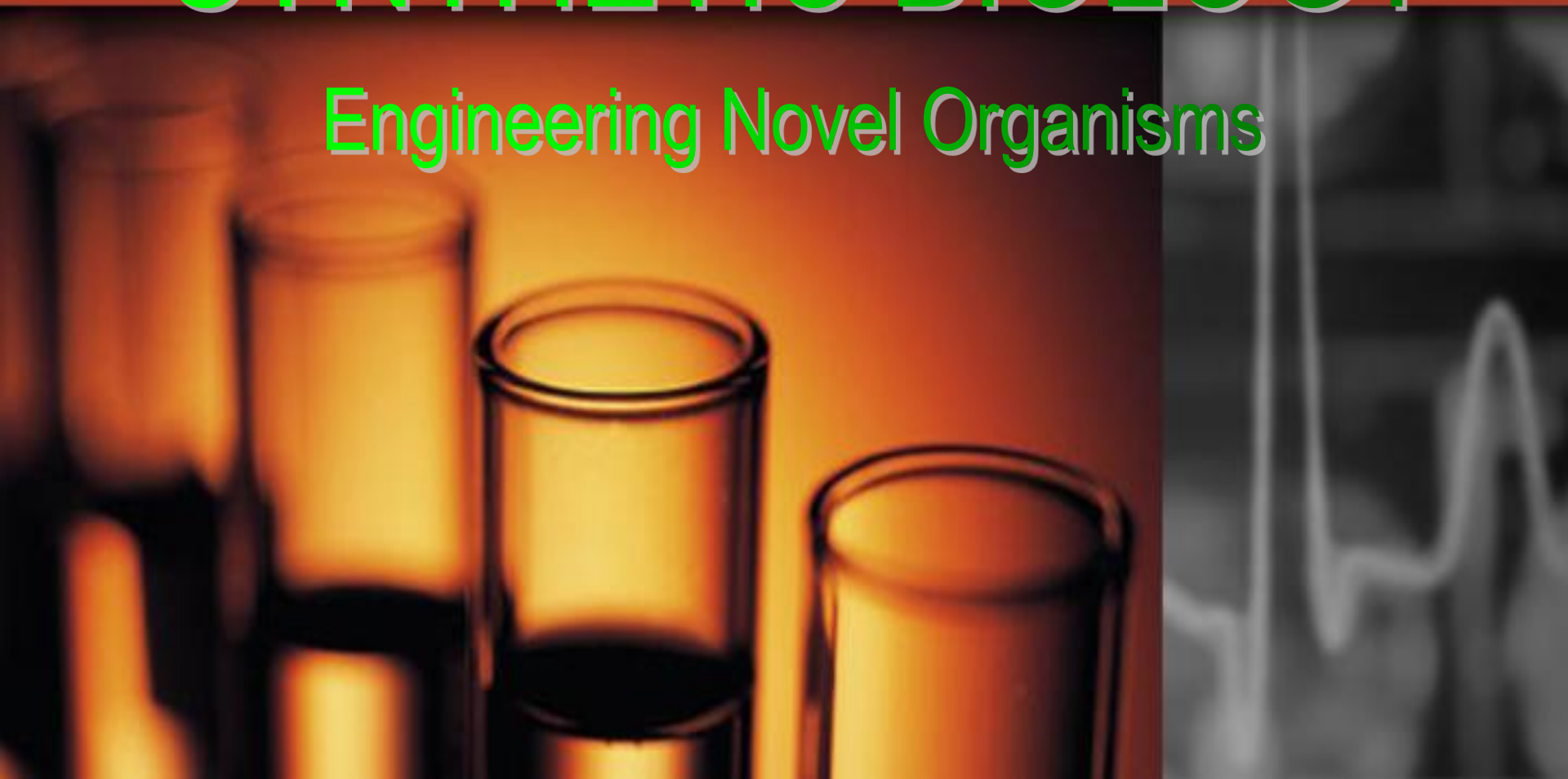
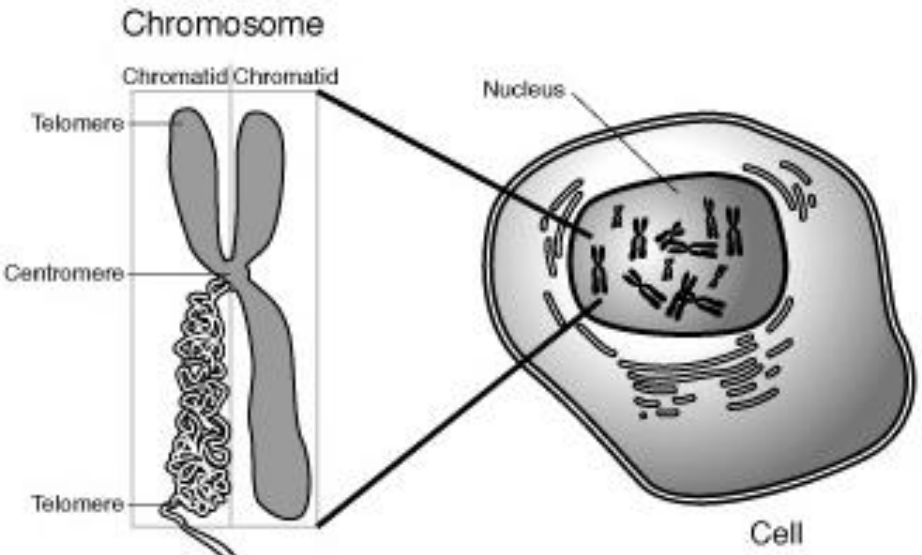
IPRO 302
SYNTHETIC BIOLOGY

Engineering Novel Organisms

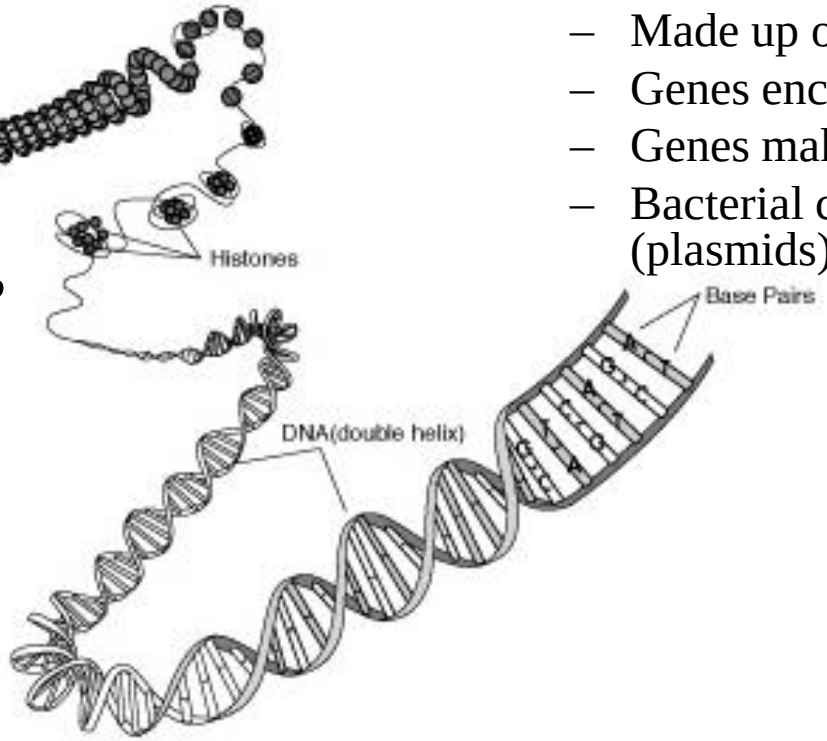


Introduction

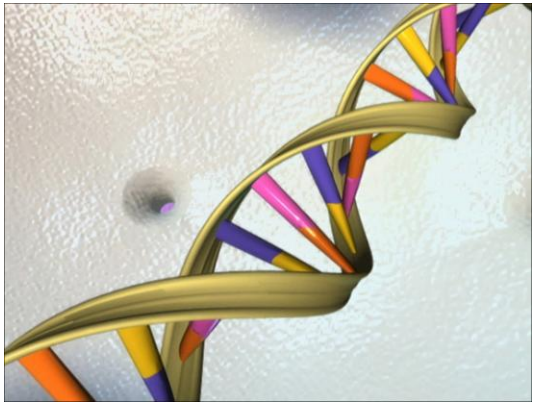
- So what is DNA, anyway?
- A long, twisted-ladder molecule
  - Contains four different nucleotide molecules (A, C, T, G).
  - Each base is a letter in the genetic code.



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

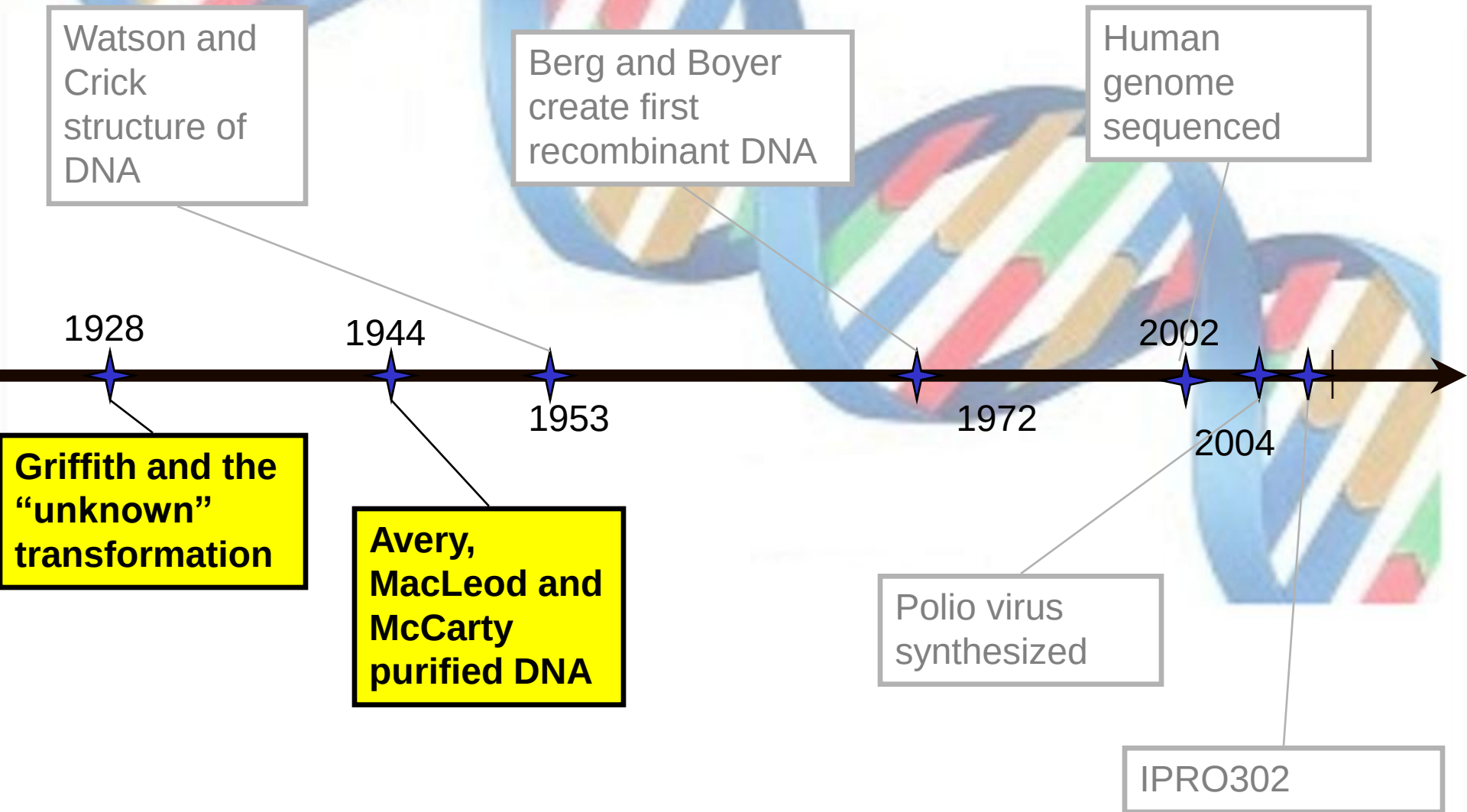


- What are we doing?
  - Manipulating bacterial genes

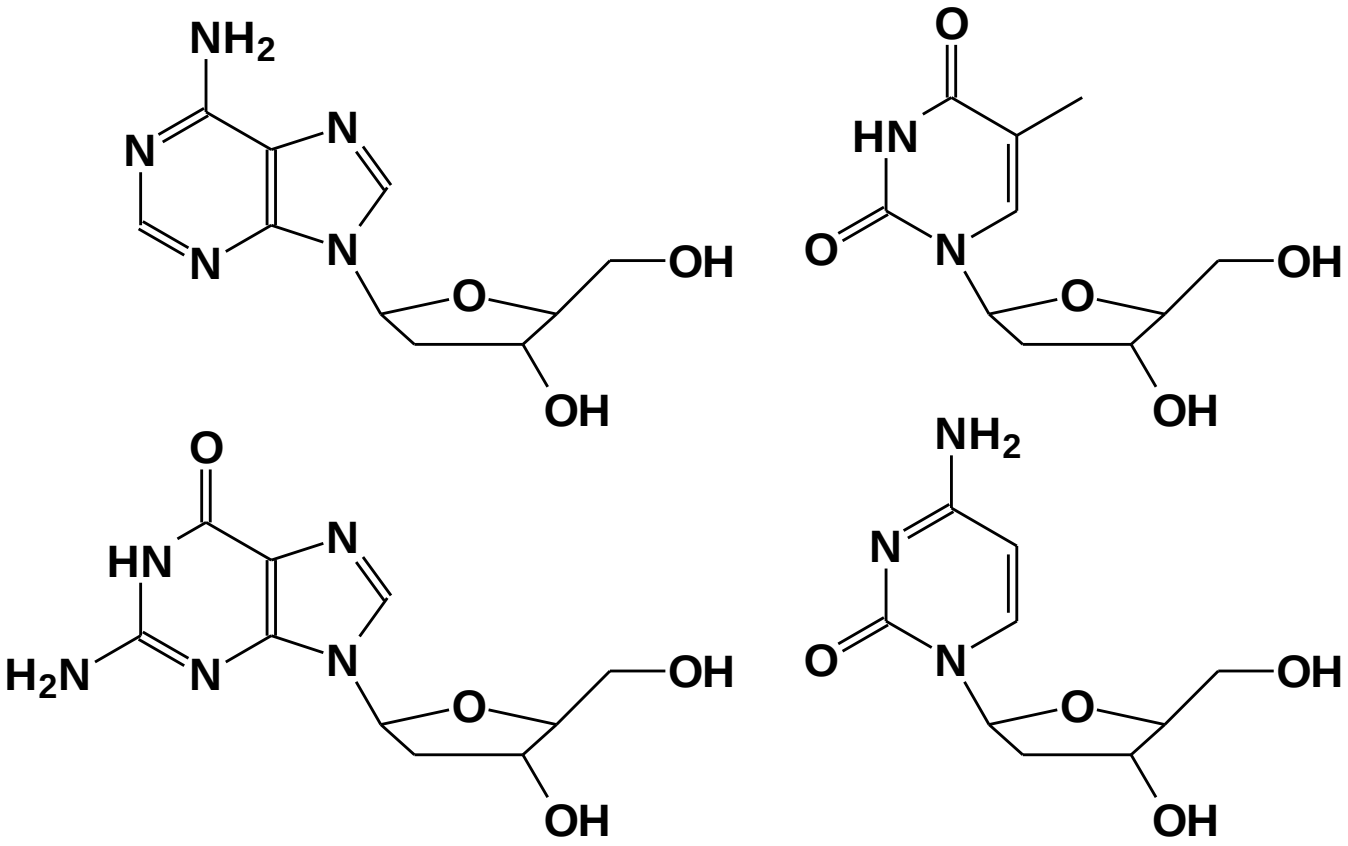


Genetics Timeline

Our view of genetics has evolved over time



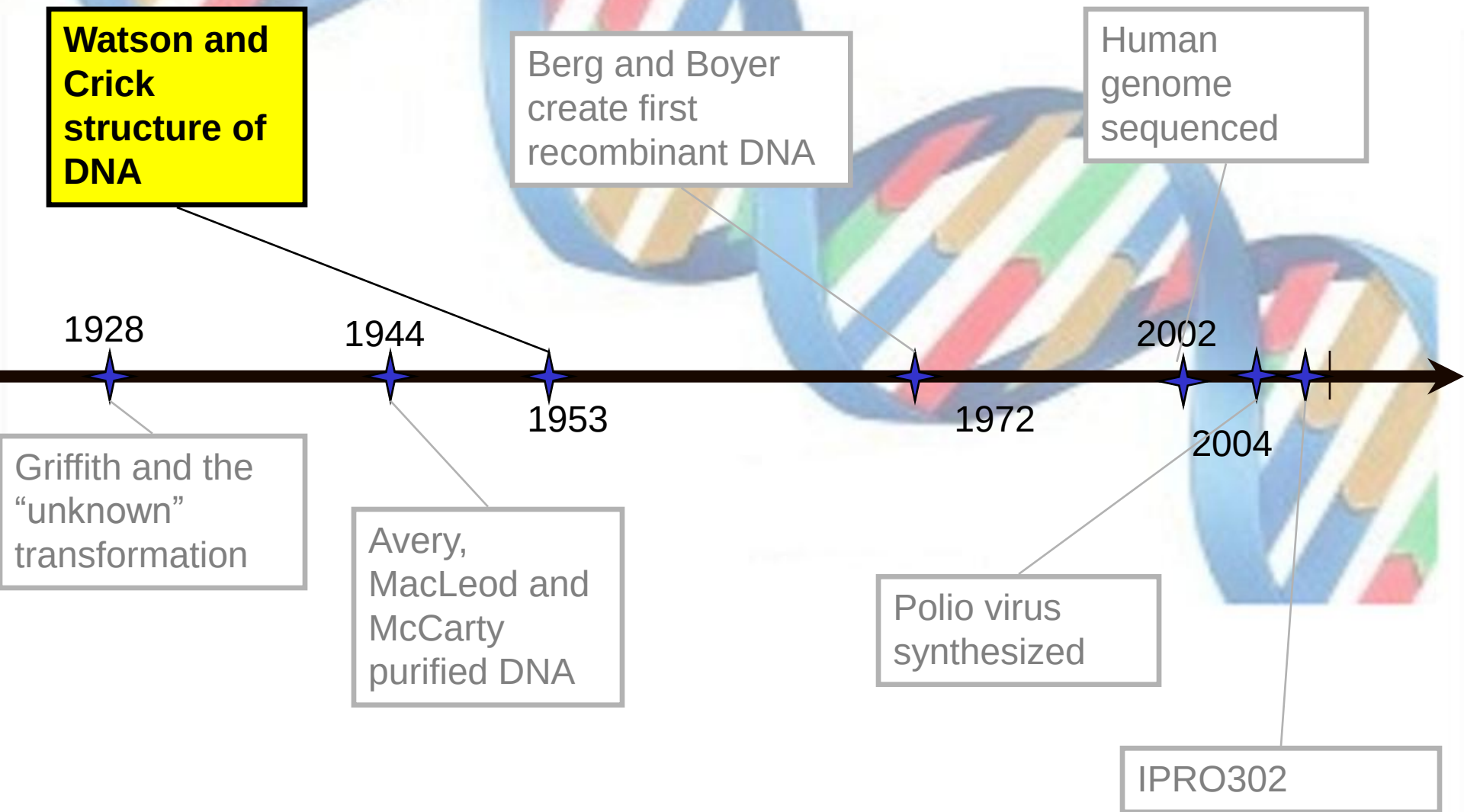
The chemicals of heredity…



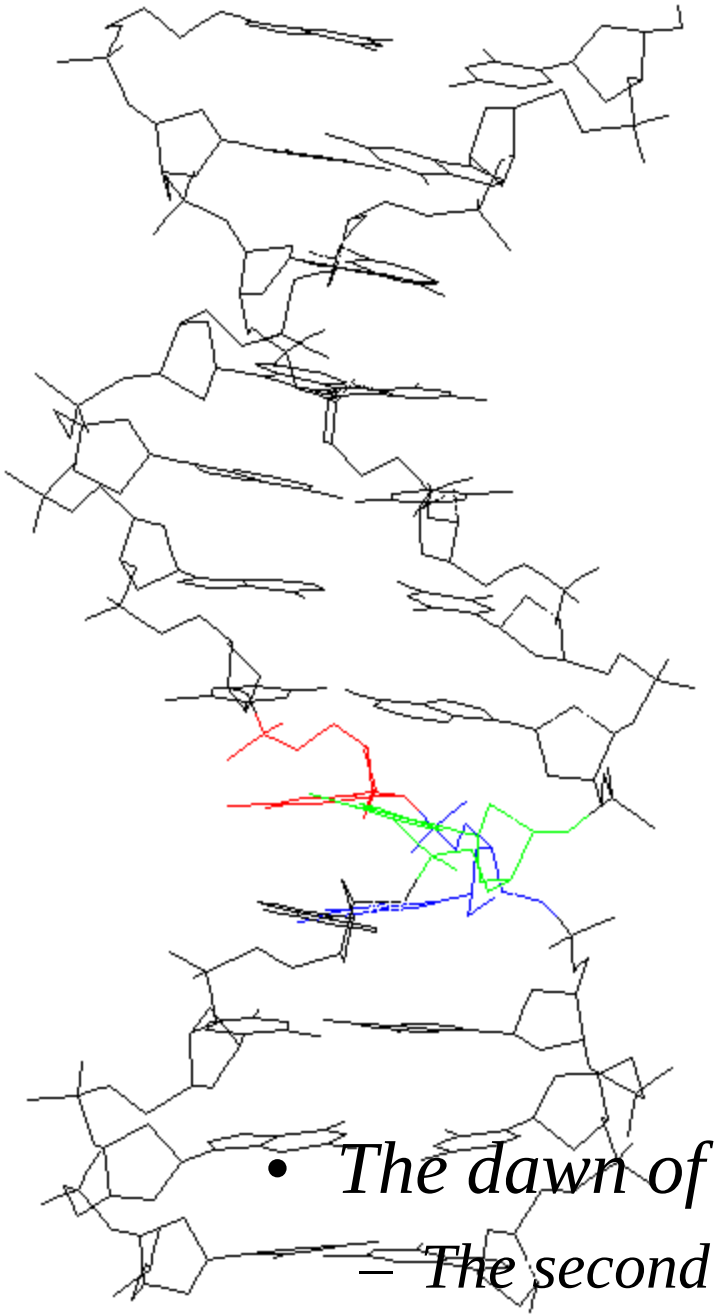
Heredity boils down to chemicals

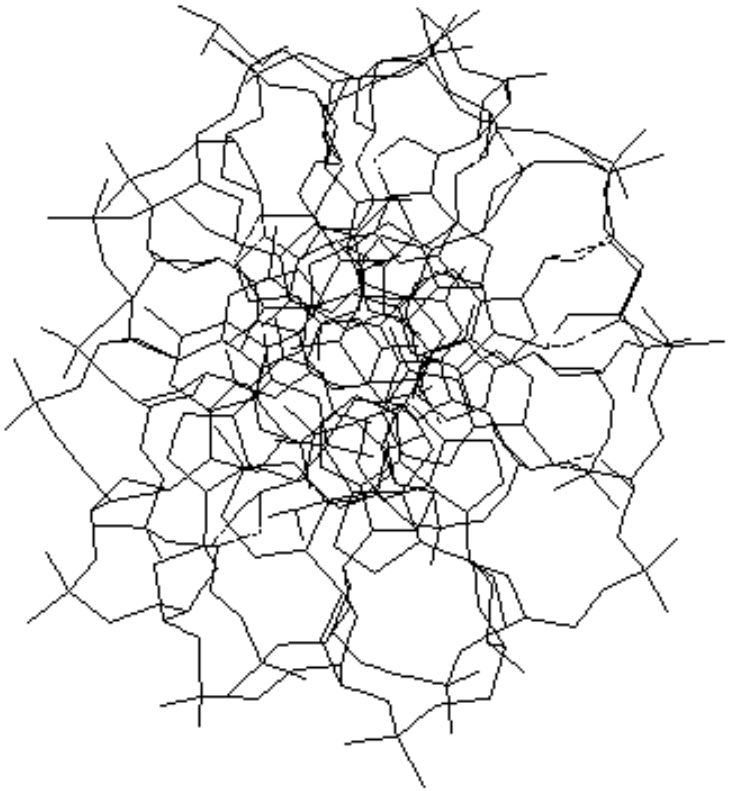
Genetics Timeline

Our view of genetics has evolved over time



DNA

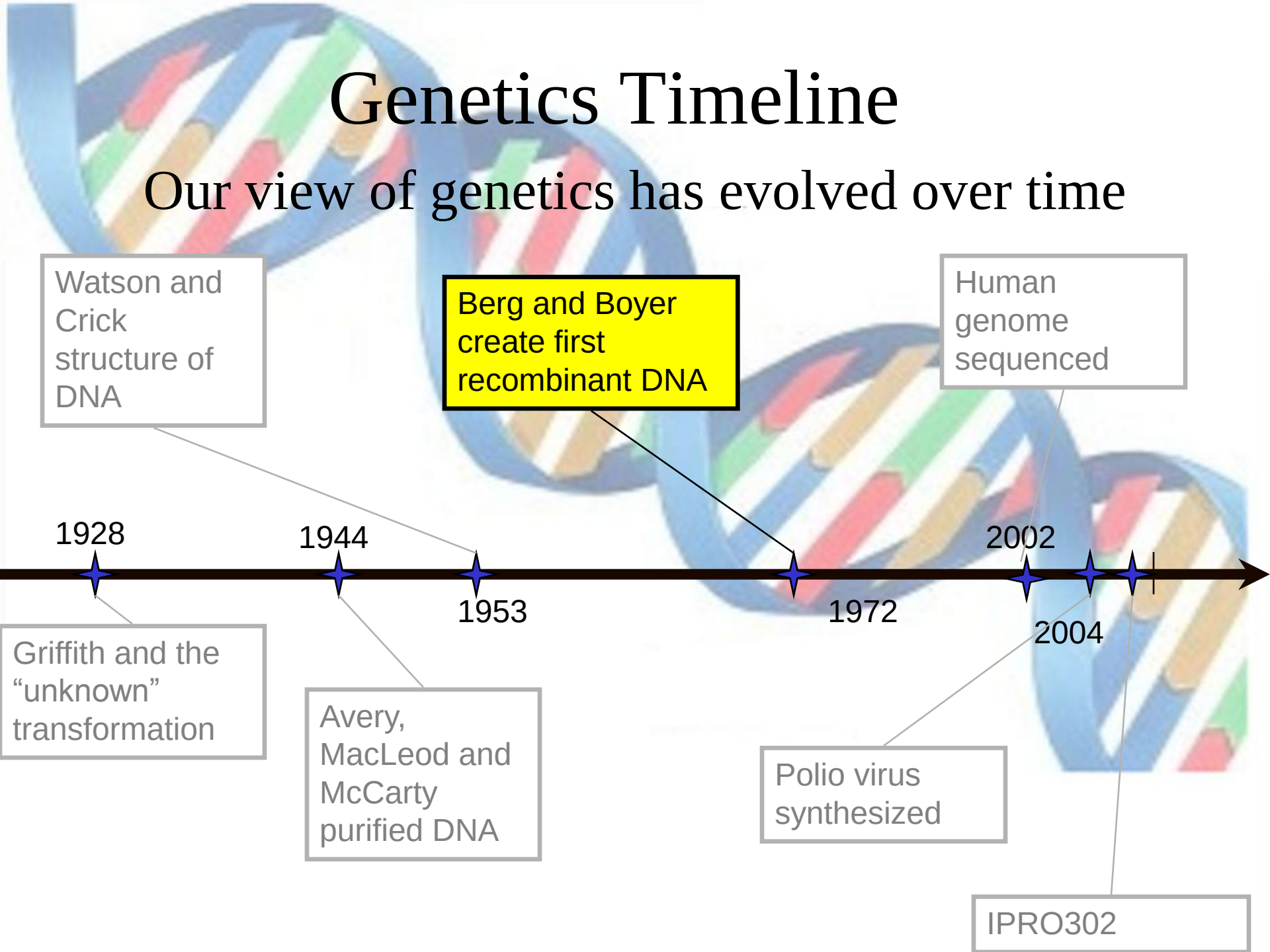




- *The dawn of the genetic era – the double helix*
  - *The second piece of the puzzle falls into place*

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 it's 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 CATTCGCCAT TCAGGCTGCG
 481 ATCCGCCCGT CGACTAACAC CGTGCGTGTT 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 ATTAAATCTC GCGCCGATCA ACTGGGTGCC
 841 AGCGTGGTGG TGTCGATGGT AGAACGAAGC GGCGTCGAAG CCTGTAAAGC GGCGGTGCAC
 901 AATCTTCTCG CGCAACGCGT CAGTGGGCTG ATCATTAACT ATCCGCTGGA TGACCAGGAT
 961 GCCATTGCTG TGGAAGCTGC CTGCACTAAT GTTCCGGCGT TATTTCTTGA TGTCTCTGAC
1021 CAGACACCCA TCAACAGTAT TATTTTCTCC CATGAAGACG GTACGCGACT GGGCGTGGAG
1081 CATCTGGTCG CATTGGGTCA CCAGCAAATC GCGCTGTTAG CGGGCCCATT AAGTTCTGTC
1141 TCGGCGCGTC TGCGTCTGGC TGGCTGGCAT AAATATCTCA CTCGCAATCA AATTCAGCCG
1201 ATAGCGGAAC GGGAAGGCGA CTGGAGTGCC ATGTCCGGTT TTCAACAAAC 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 AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATGCAGCT GGCACGACAG
1621 GTTTCCCGAC TGGAAAGCGG GCAGGCAGCA AATGATGAGA ATTATGCAGC AGCTGTATAA
1681 GCGGCCGCAA AAAACCCCTC AAGACCCGTT TAGAGGCCCC AAGGGGTTAT GCTACTTAAG
1741 GGGCTAGAGC GGCCCATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC
1801 GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC
```

It starts with letters

Words…

```
 421 CACACCCGCC GCGCTTAATG CGCCGCTACA GGGCGCGTCC CATTCGCCAT TCAGGCTGCG
 481 ATCCGCCCGT CGACTAACAC CGTGCGTGTT 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 ATTAAATCTC GCGCCGATCA ACTGGGTGCC
 841 AGCGTGGTGG TGTCGATGGT AGAACGAAGC GGCGTCGAAG CCTGTAAAGC GGCGGTGCAC
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 961 GCCATTGCTG TGGAAGCTGC CTGCACTAAT GTTCCGGCGT TATTTCTTGA TGTCTCTGAC
1021 CAGACACCCA TCAACAGTAT TATTTTCTCC CATGAAGACG GTACGCGACT GGGCGTGGAG
1081 CATCTGGTCG CATTGGGTCA CCAGCAAATC GCGCTGTTAG CGGGCCCATT AAGTTCTGTC
1141 TCGGCGCGTC TGCGTCTGGC TGGCTGGCAT AAATATCTCA CTCGCAATCA AATTCAGCCG
1201 ATAGCGGAAC GGGAAGGCGA CTGGAGTGCC ATGTCCGGTT TTCAACAAAC CATGCAAATG
1261 CTGAATGAGG GCATCGTTCC CACTGCGATG CTGGTTGCCA ACGATCAGAT GGCGCTGGGC
1321 GCAATGCGCG CCATTACCGA GTCCGGGCTG CGCGTTGGTG CGGATATCTC GGTAGTGGGA
1381 TACGACGATA CCGAAGACAG CTCATGTTAT ATCCCGCCGT TAACCACCAT CAAACAGGAT
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1501 GTGAAGGGCA ATCAGCTGTT GCCCGTCTCA CTGGTGAAAA GAAAAACCAC CCTGGCGCCC
1561 AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT TAATGCAGCT GGCACGACAG
1621 GTTTCCCGAC TGGAAAGCGG GCAGGCAGCA AATGATGAGA ATTATGCAGC AGCTGTATAA
1681 GCGGCCGCAA AAAACCCCTC AAGACCCGTT TAGAGGCCCC AAGGGGTTAT GCTACTTAAG
1741 GGGCTAGAGC GGCCCATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC
1801 GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC
```

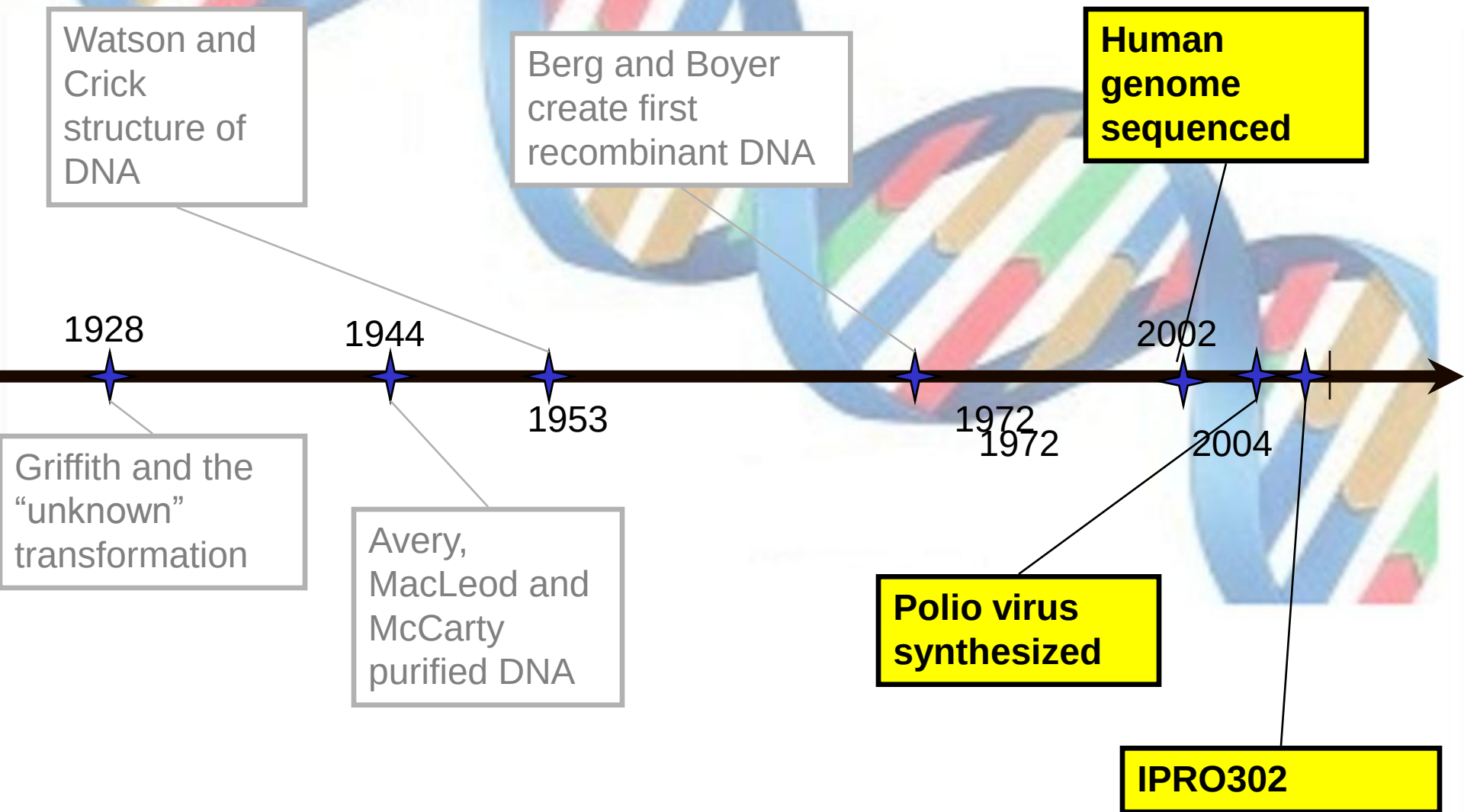
Then we progress to words

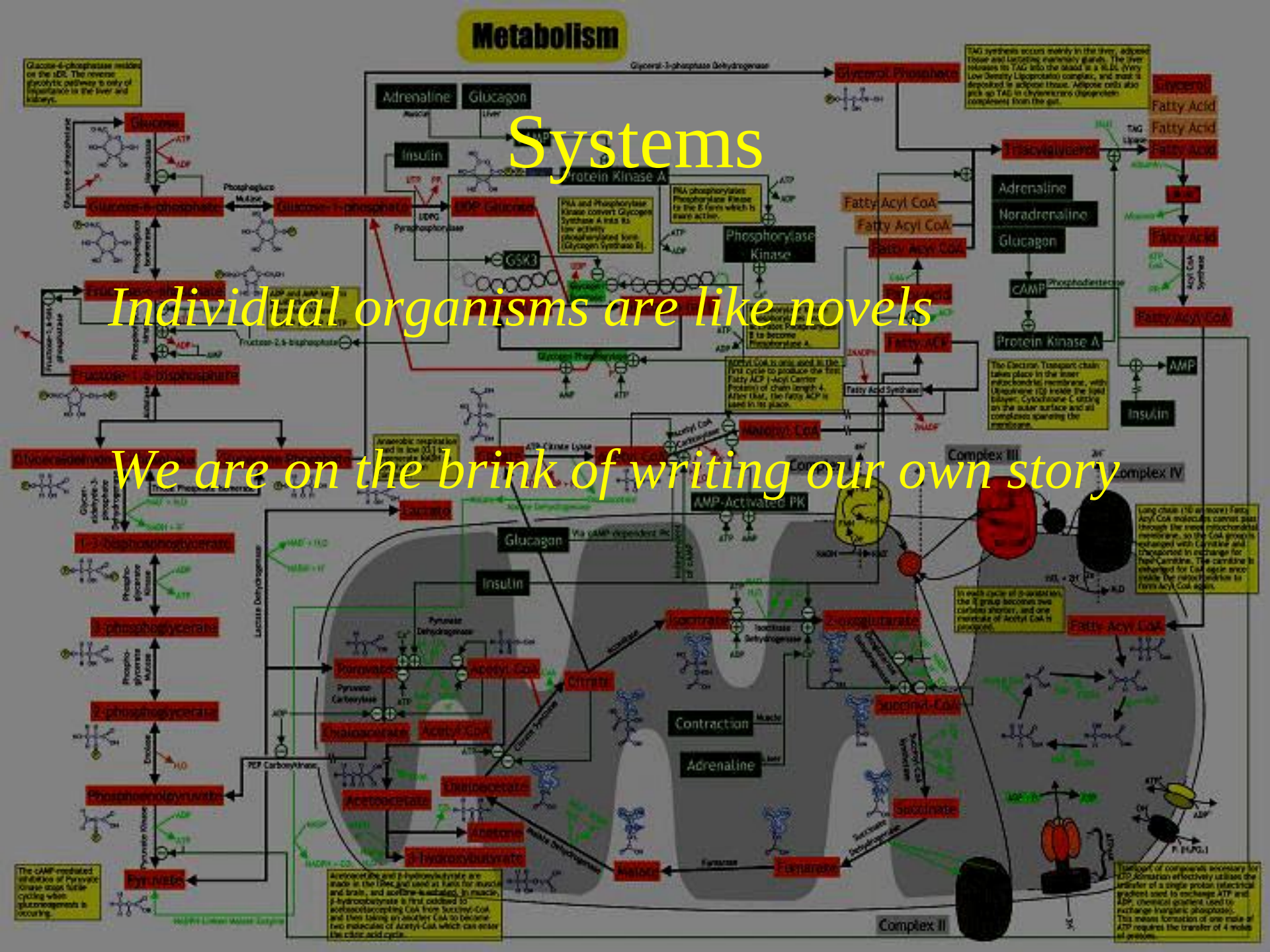
Meanings

And then we go to paragraphs…

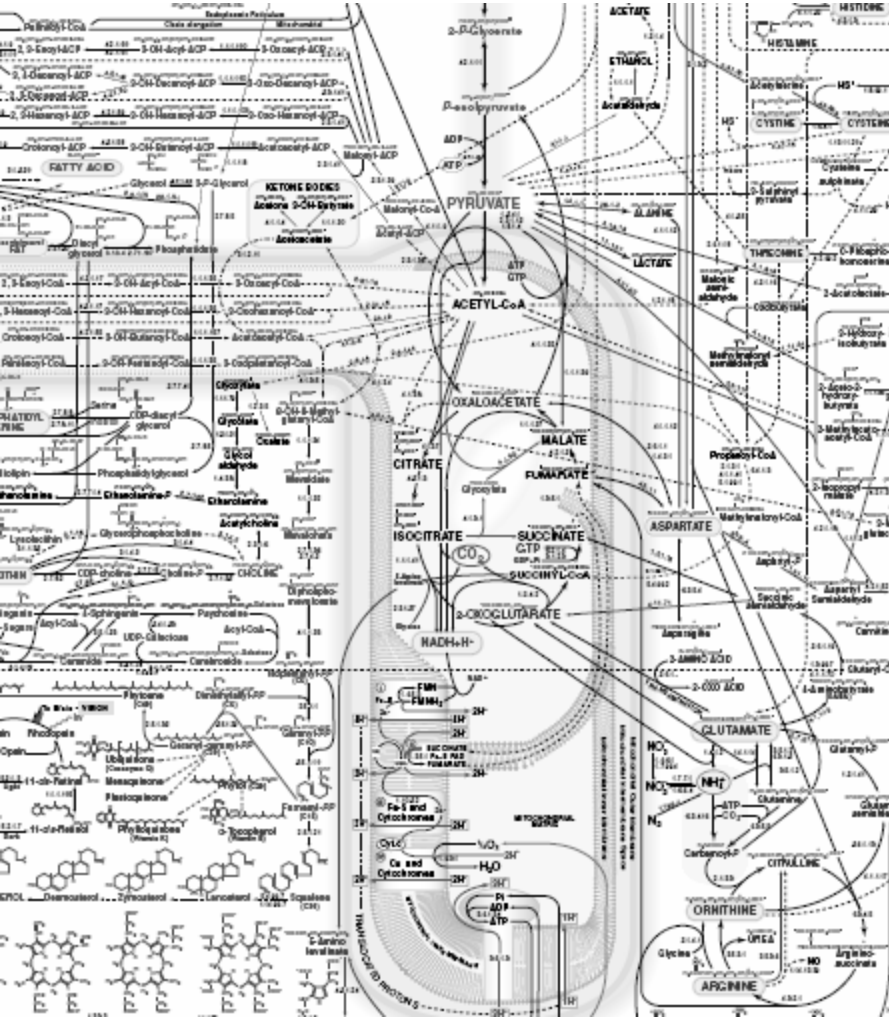
Genetics Timeline

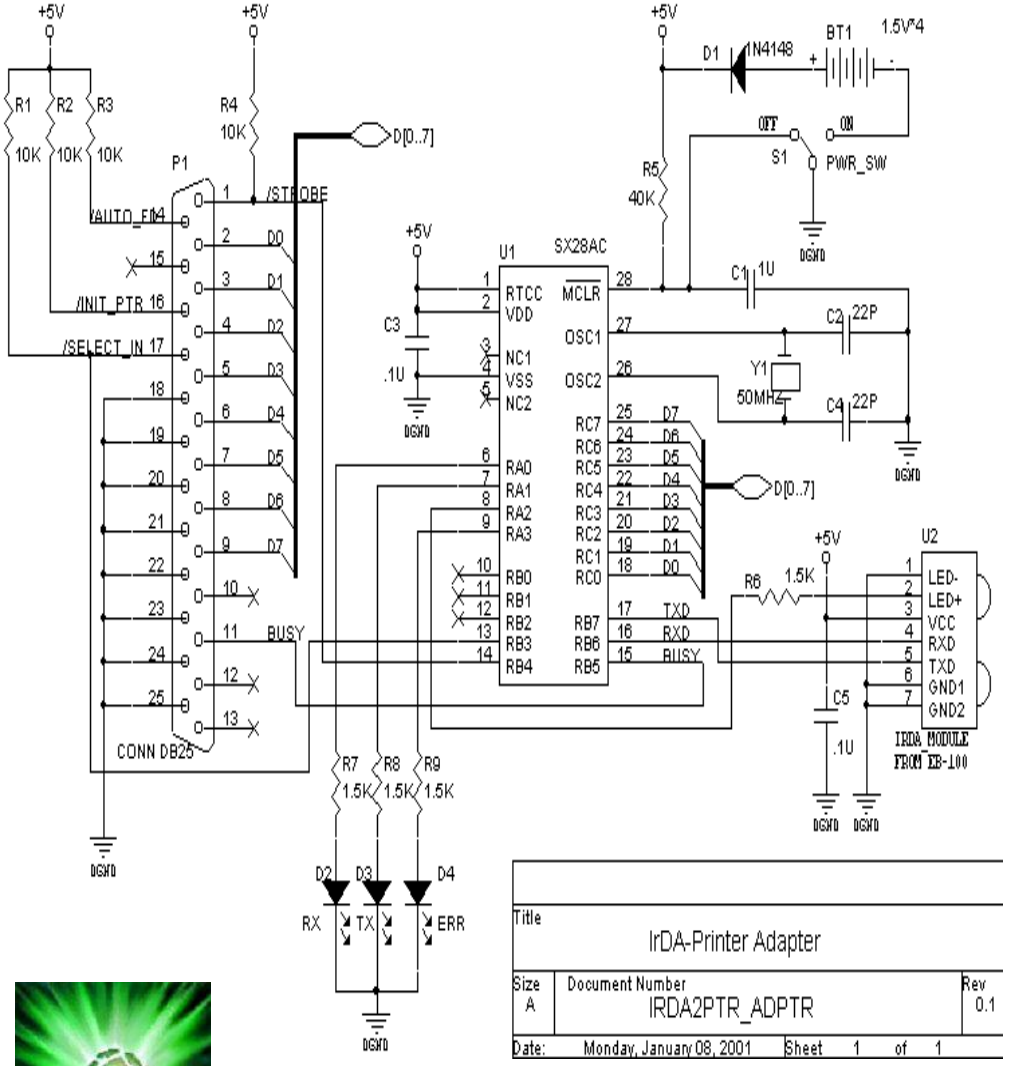
Our view of genetics has evolved over time




Metabolism
Systems
Individual organisms are like novels
We are on the brink of writing our own story

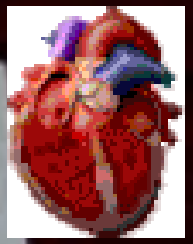
Biology and Engineering Collide





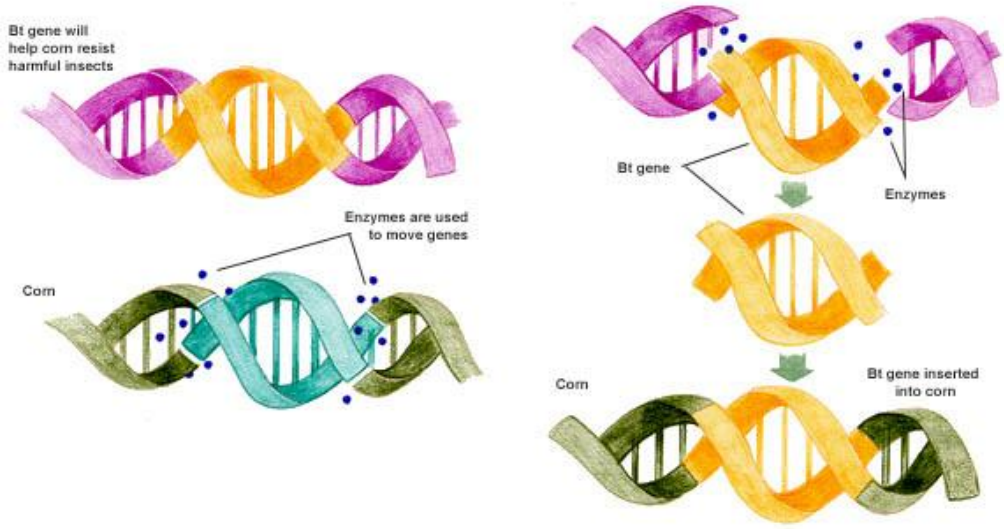
Metabolic Pathways → ← Circuits Schematic

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?**

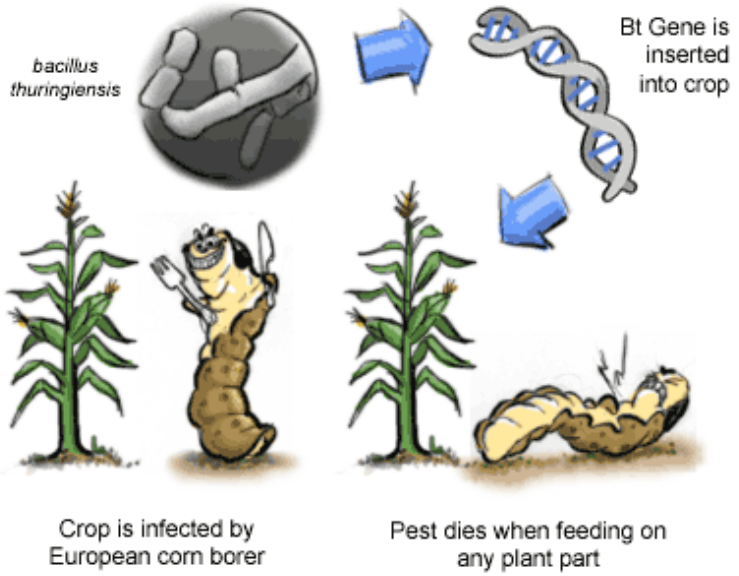
Our Project Goal



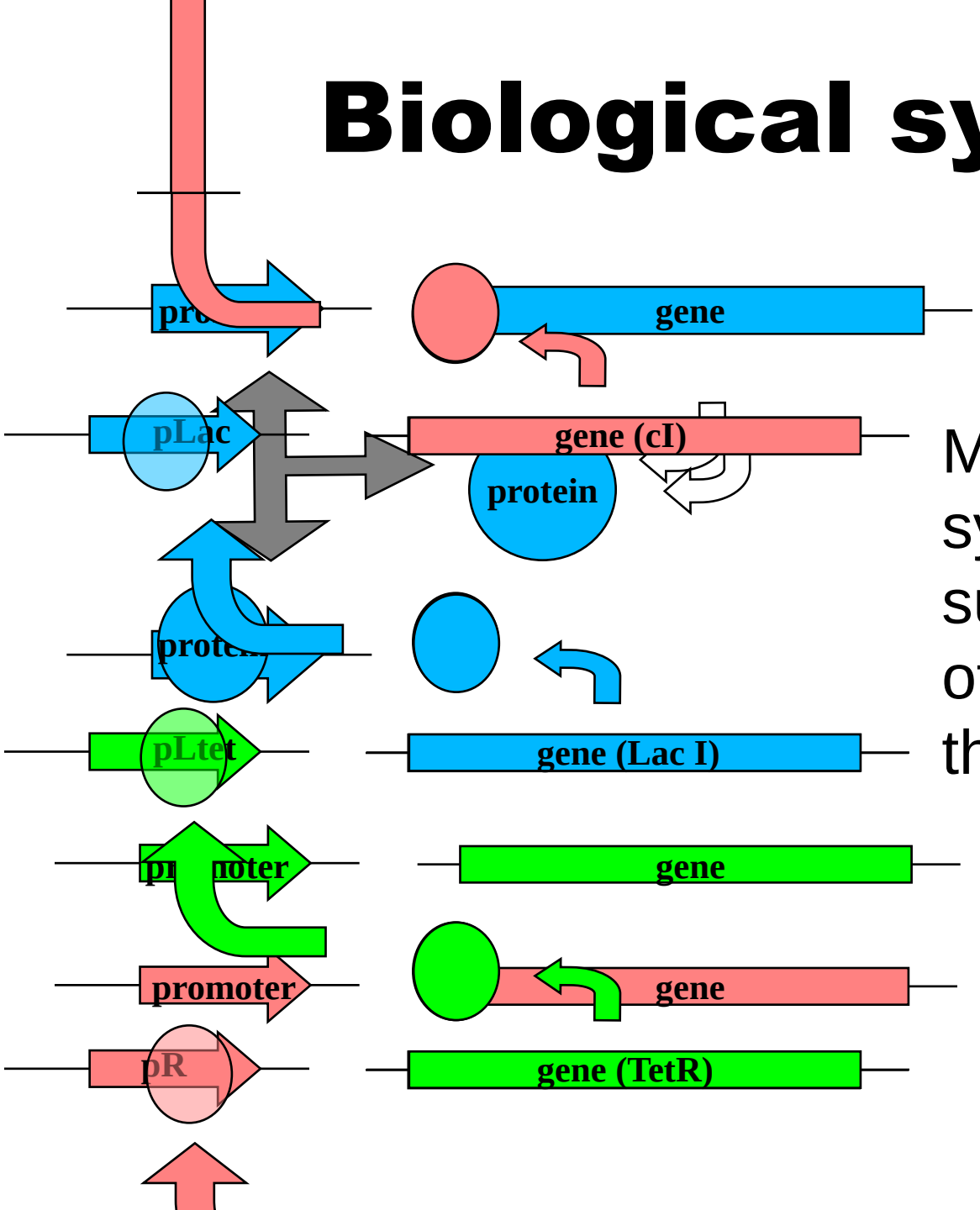
- Design a genetic oscillating system by modifying and combining existing gene systems

- Inserting a single feature in an organism has become common practice

- Our projects goal is to combine features together and create a novel behavior



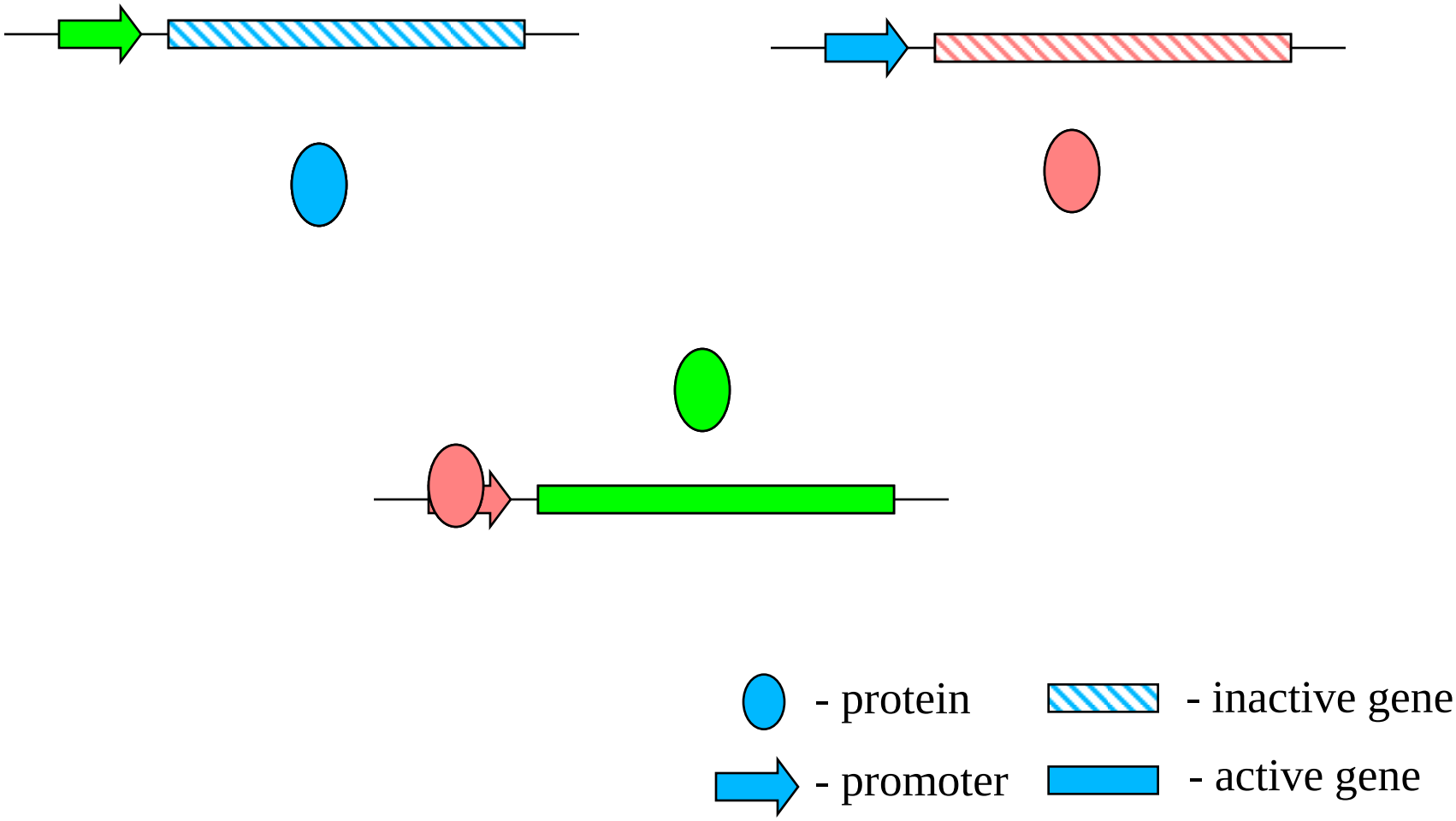
Biological system



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

From Biology to Engineering

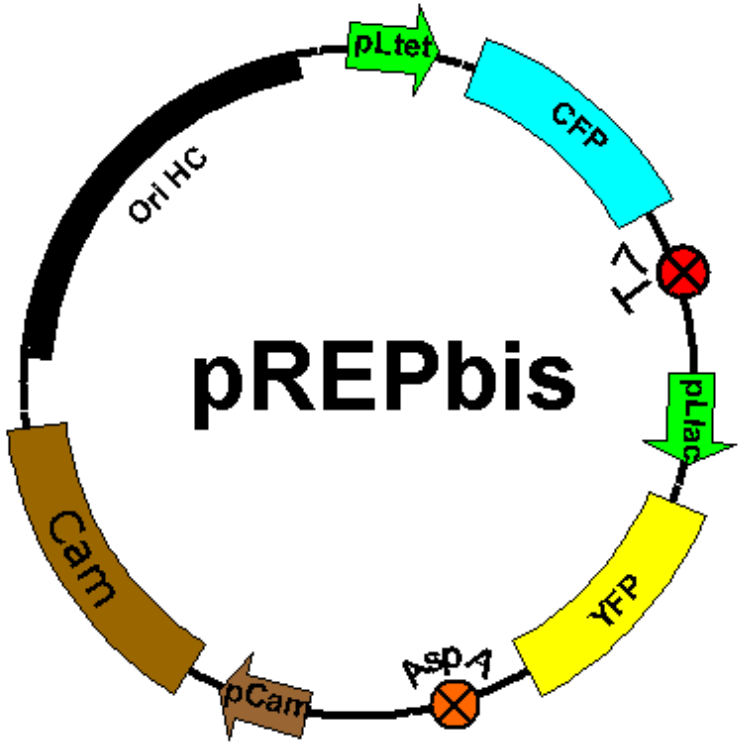
Rearrange gene pieces to obtain an oscillating system – how it all works together?



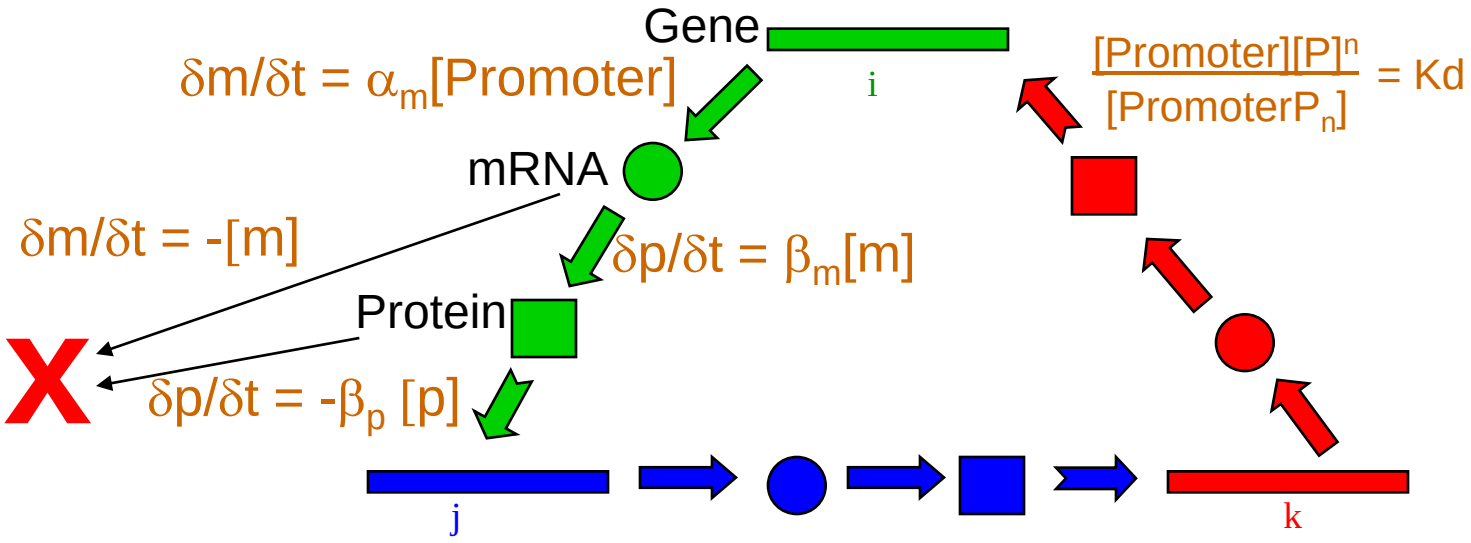
Reporter plasmid

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:

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

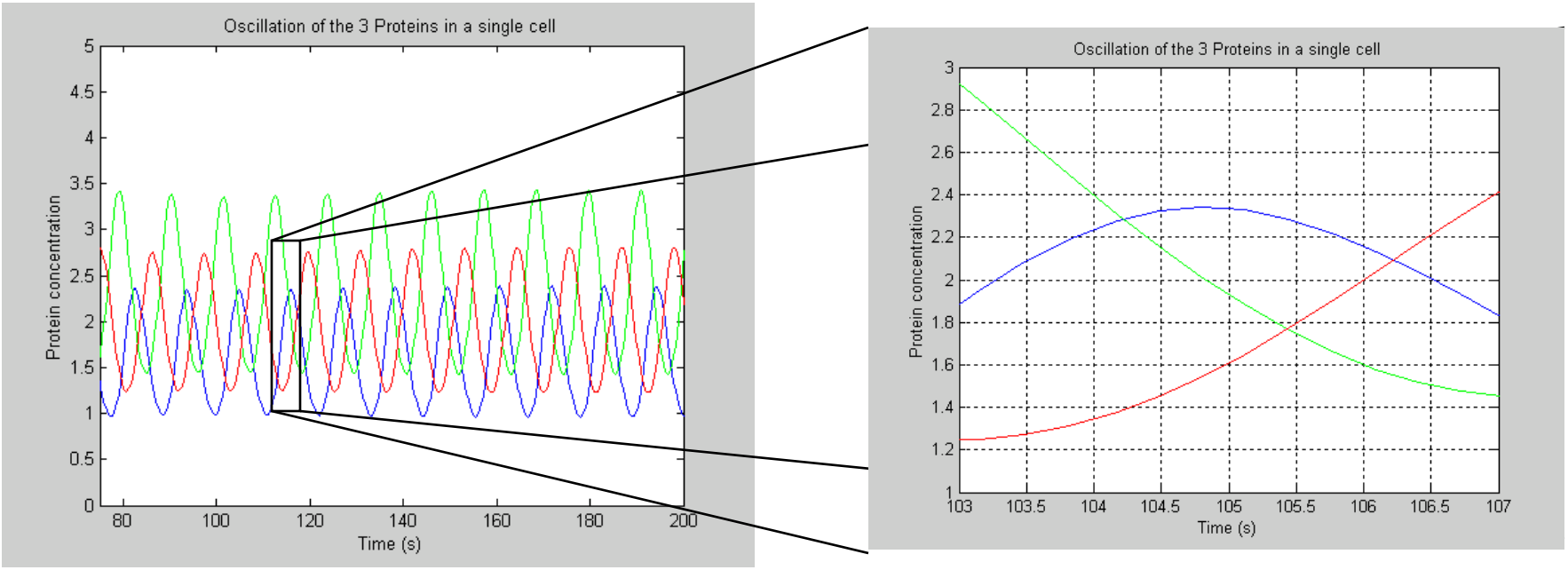
$$\frac{dp_j}{dt} = -\beta(p_j - m_j)$$

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

Matlab

Promoter	replication origin of Vector	copy number in log Phase	Promoter strength (RLU/cell x 10^{-4}) -aTc	+aTc	Regulatory range
	ColE1	50 - 70	11	27900	2535
$P_{LtetO-1}$	p15A	20 - 30	3,5	12850	3670
	pSC101*	3 - 4	0,4	2020	5050
			-IPTG	+IPTG	
$P_{LlacO-1}$	ColE1	50 - 70	35	21630	620
$P_{A1lacO-1}$	ColE1	50 - 70	30	10430	350

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



Progress

Fall 2004:

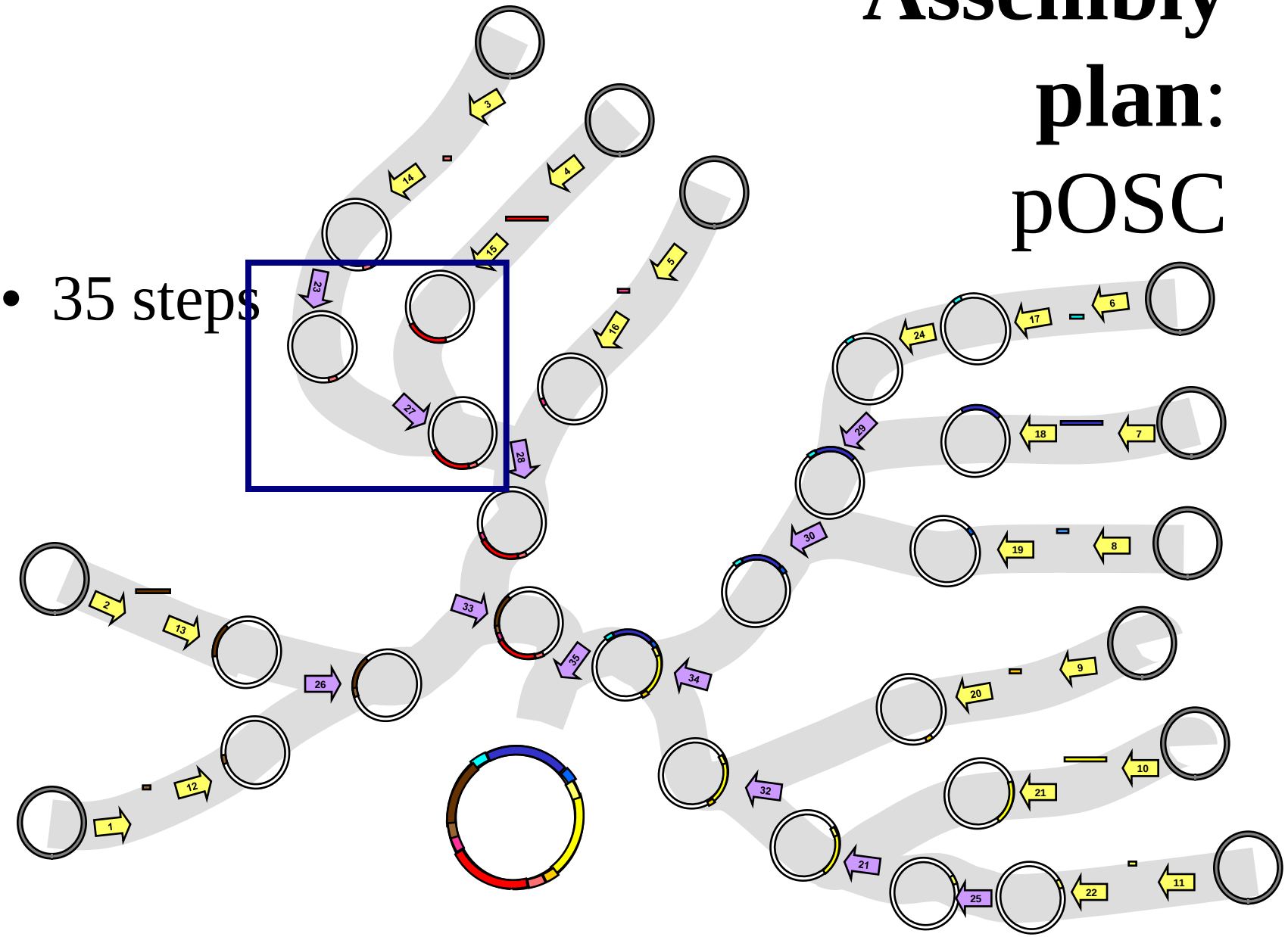
- Genes were located and ordered
- Mathematical modeling was preformed

Spring 2005:

- Gene Bank was created

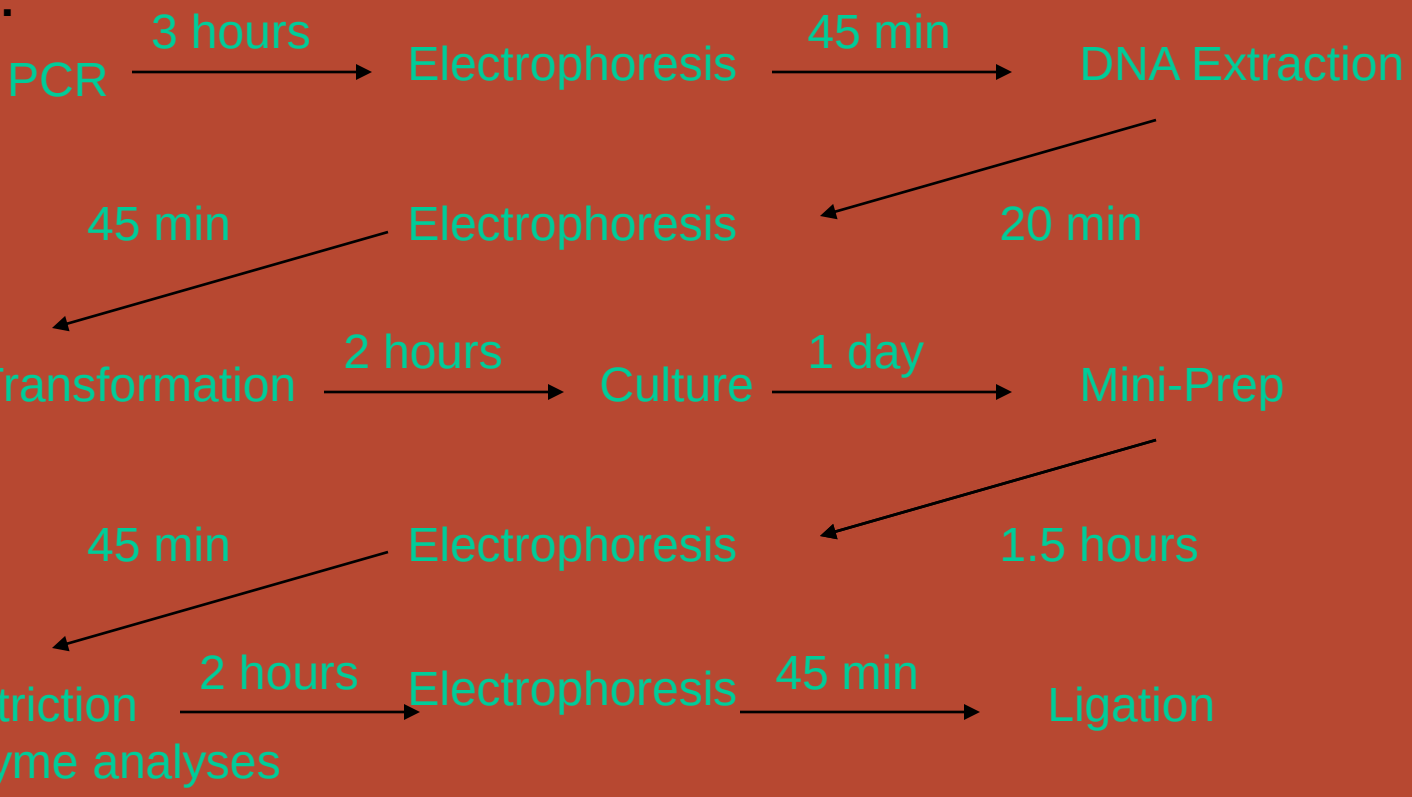
Assembly plan: pOSC

- 35 steps

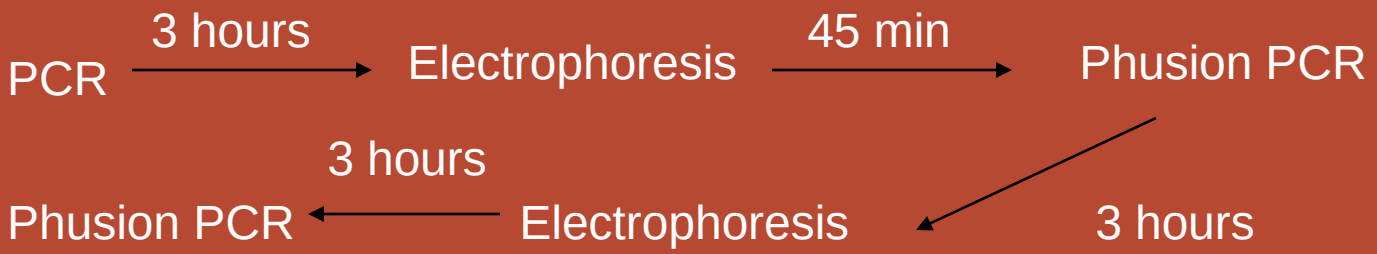


Fall 2005: Project revisions

Spring:



Fall:



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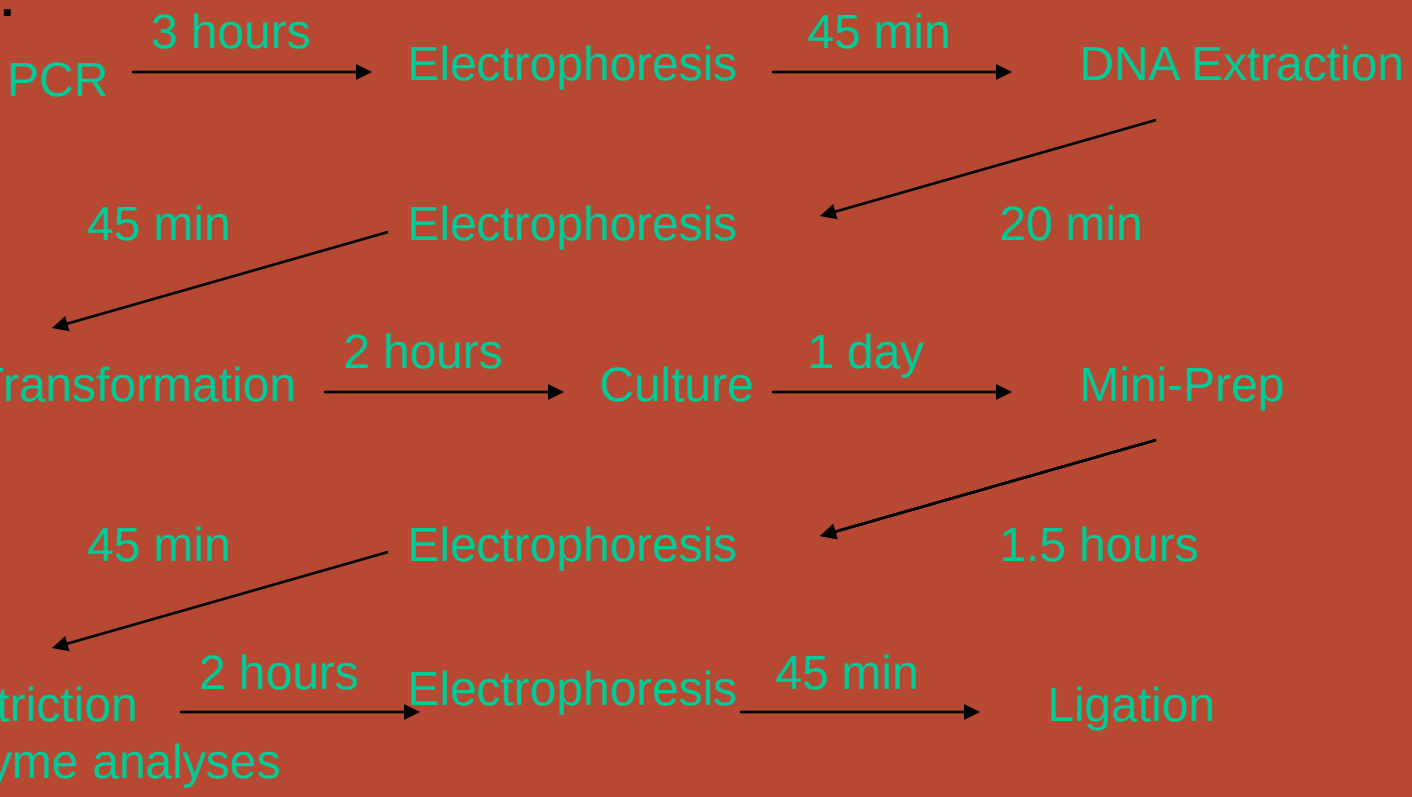

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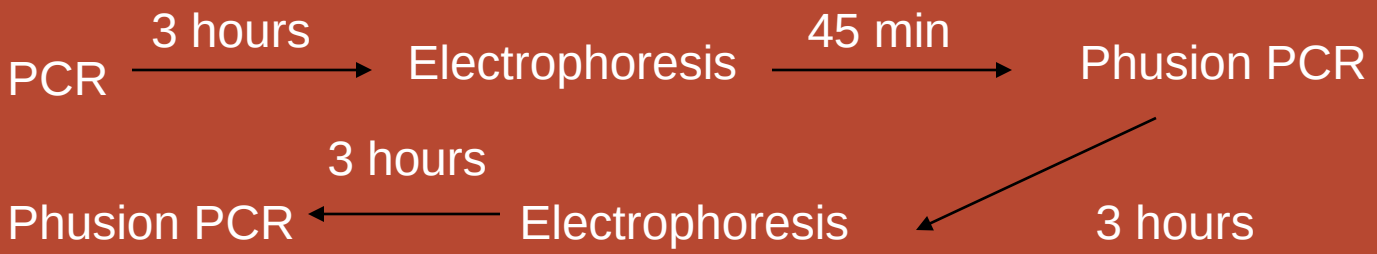
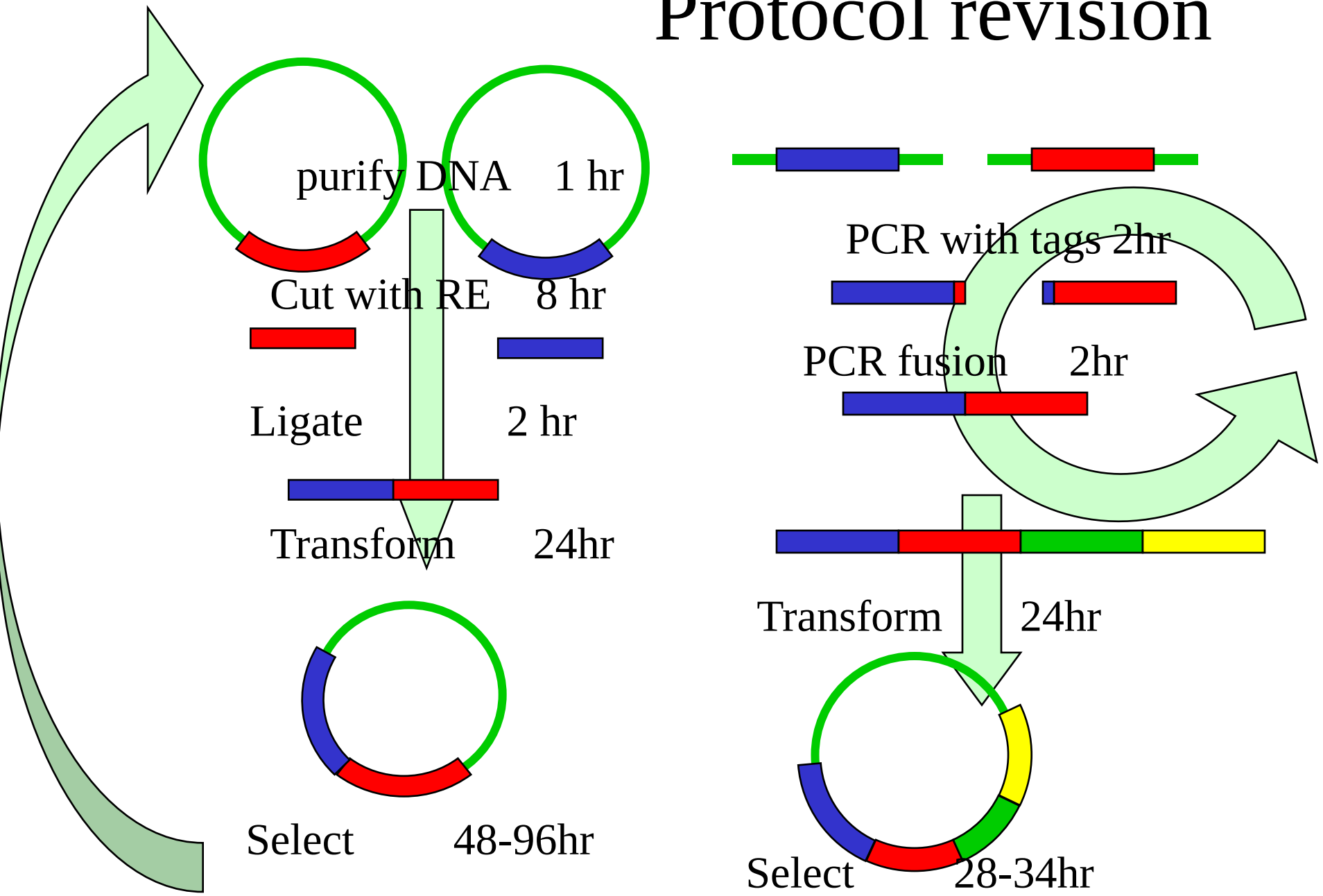


Protocol revision



Results

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

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	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.

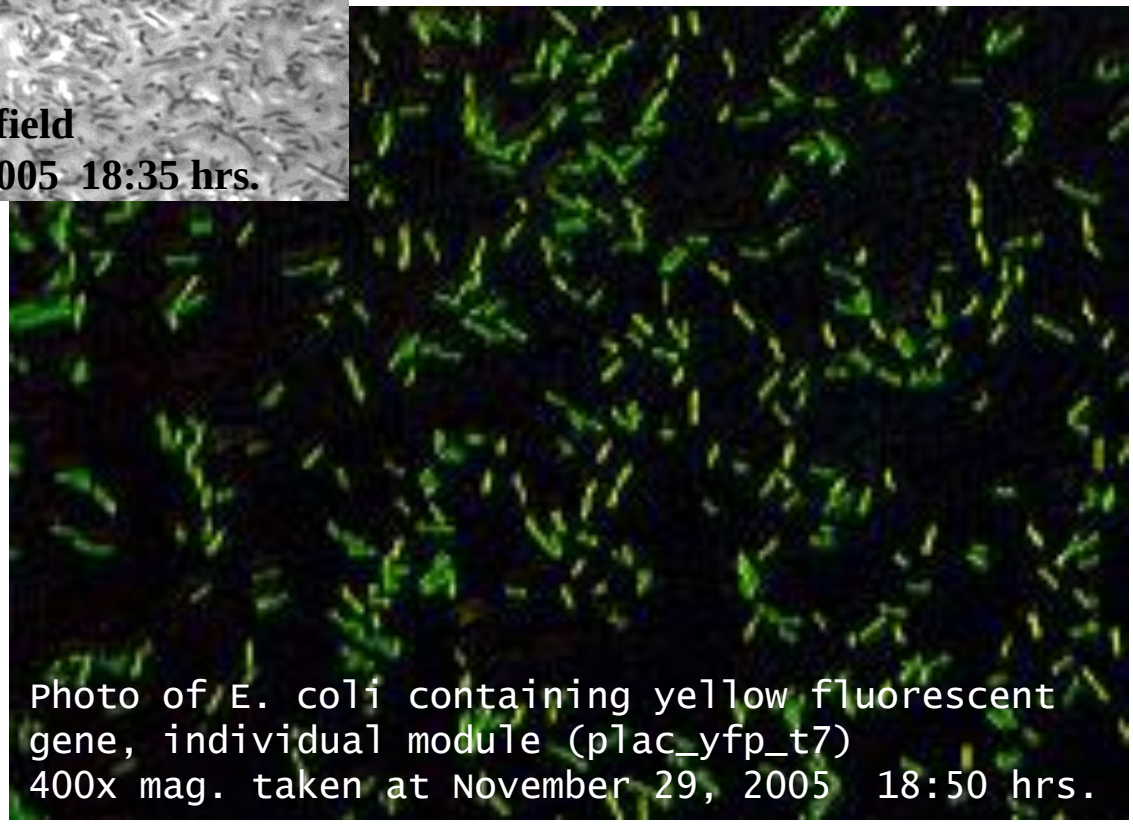


Photo of E. coli containing yellow fluorescent gene, individual module (plac_yfp_t7)
400x mag. taken at November 29, 2005 18:50 hrs.

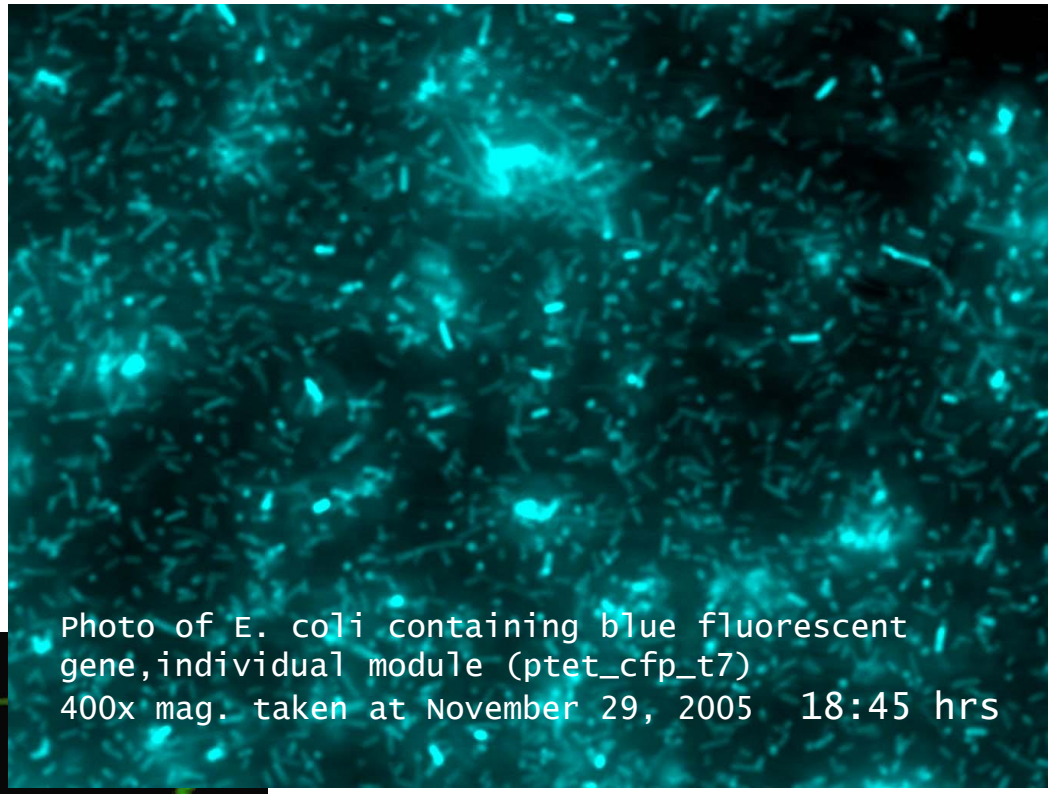


Photo of E. coli containing blue fluorescent gene,individual module (ptet_cfp_t7)
400x mag. taken at November 29, 2005 18:45 hrs

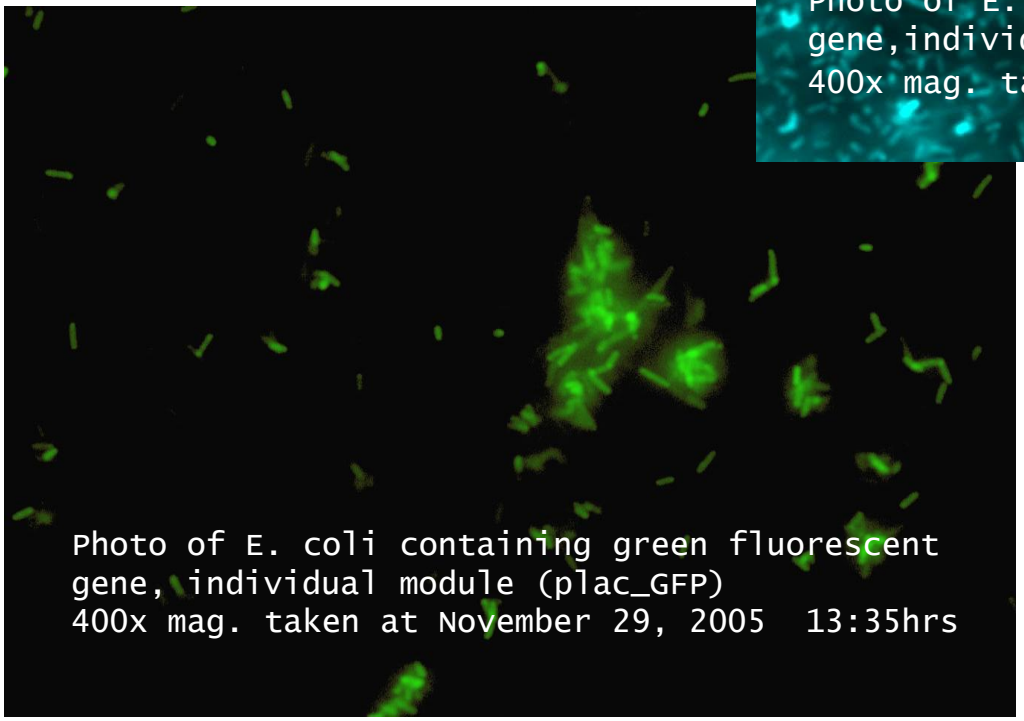
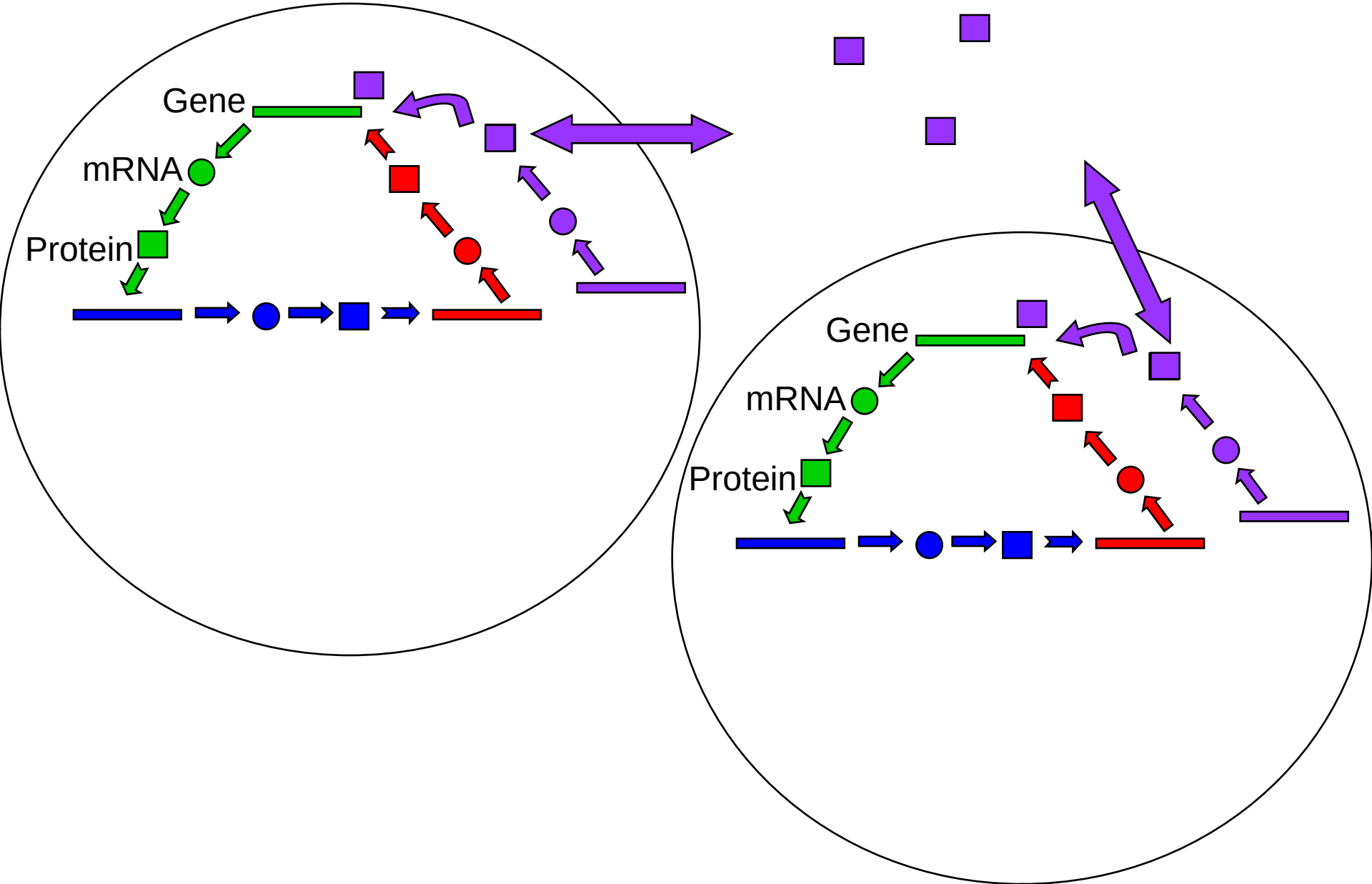


Photo of E. coli containing green fluorescent gene, individual module (plac_GFP)
400x mag. taken at November 29, 2005 13:35hrs

Synchronization



Modeling

mRNA Equations:

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

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

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

Protein Equations:

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

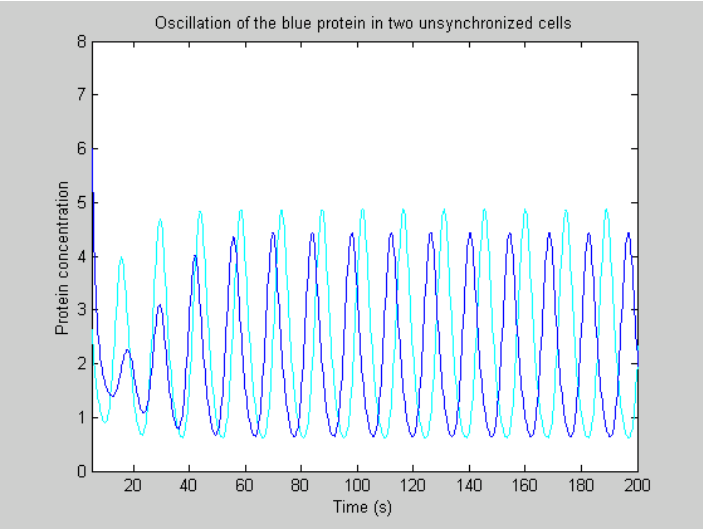
$$\frac{dp_j}{dt} = -\beta(p_j - m_j)$$

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

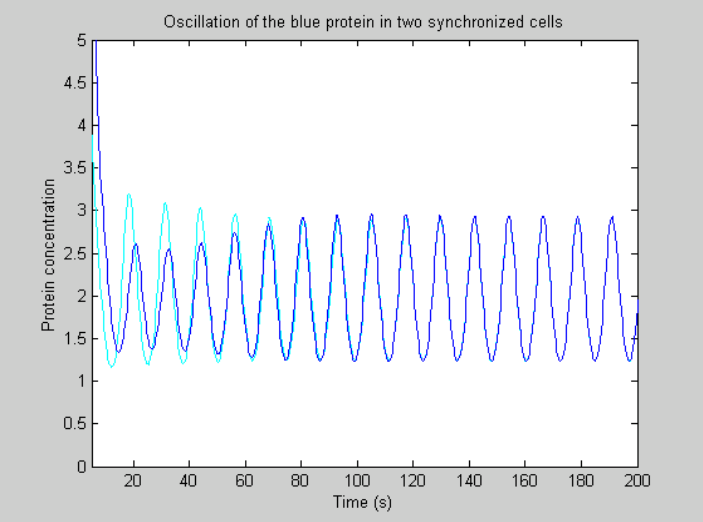
$$\frac{dS_{int}}{dt} = -k_{s0}S_{int} + k_{s1}p_k - \eta(S_{int} - S_{ext})$$

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

Matlab

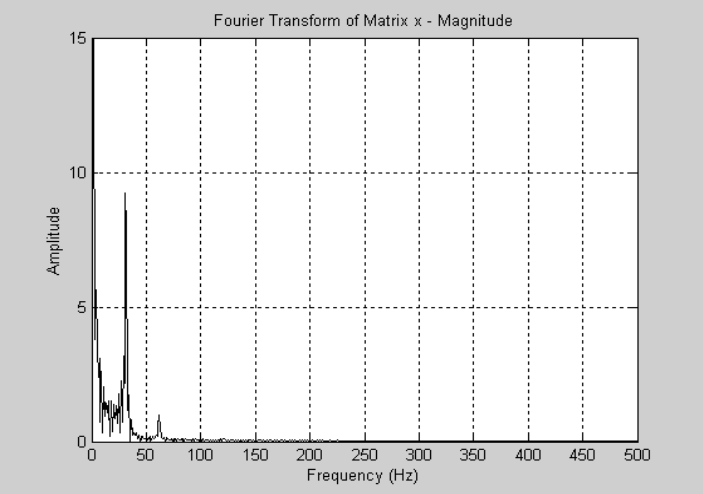


Unsynchronized

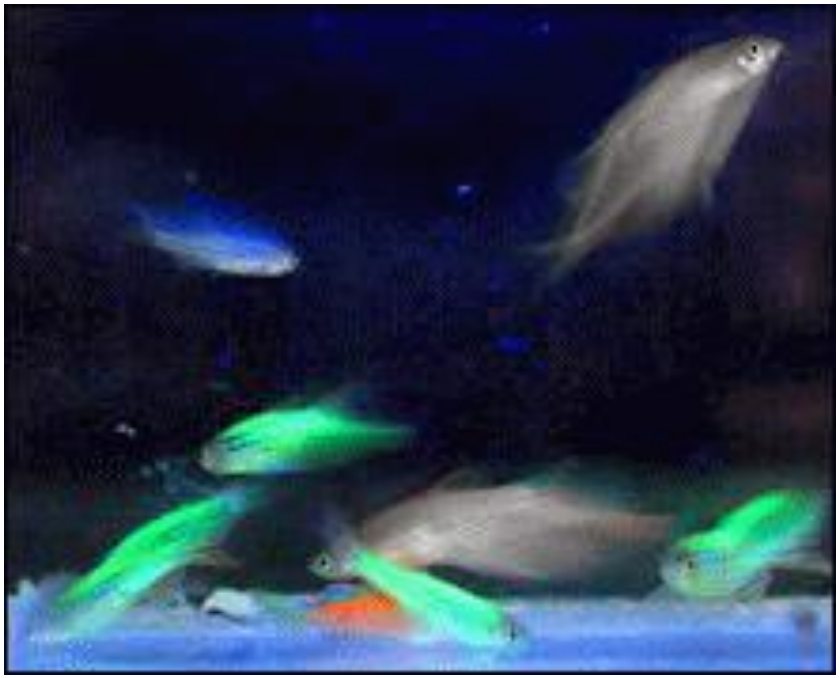


Synchronized !

Fourier Transform



Future Work



Danio rerio

Application to vertebrates

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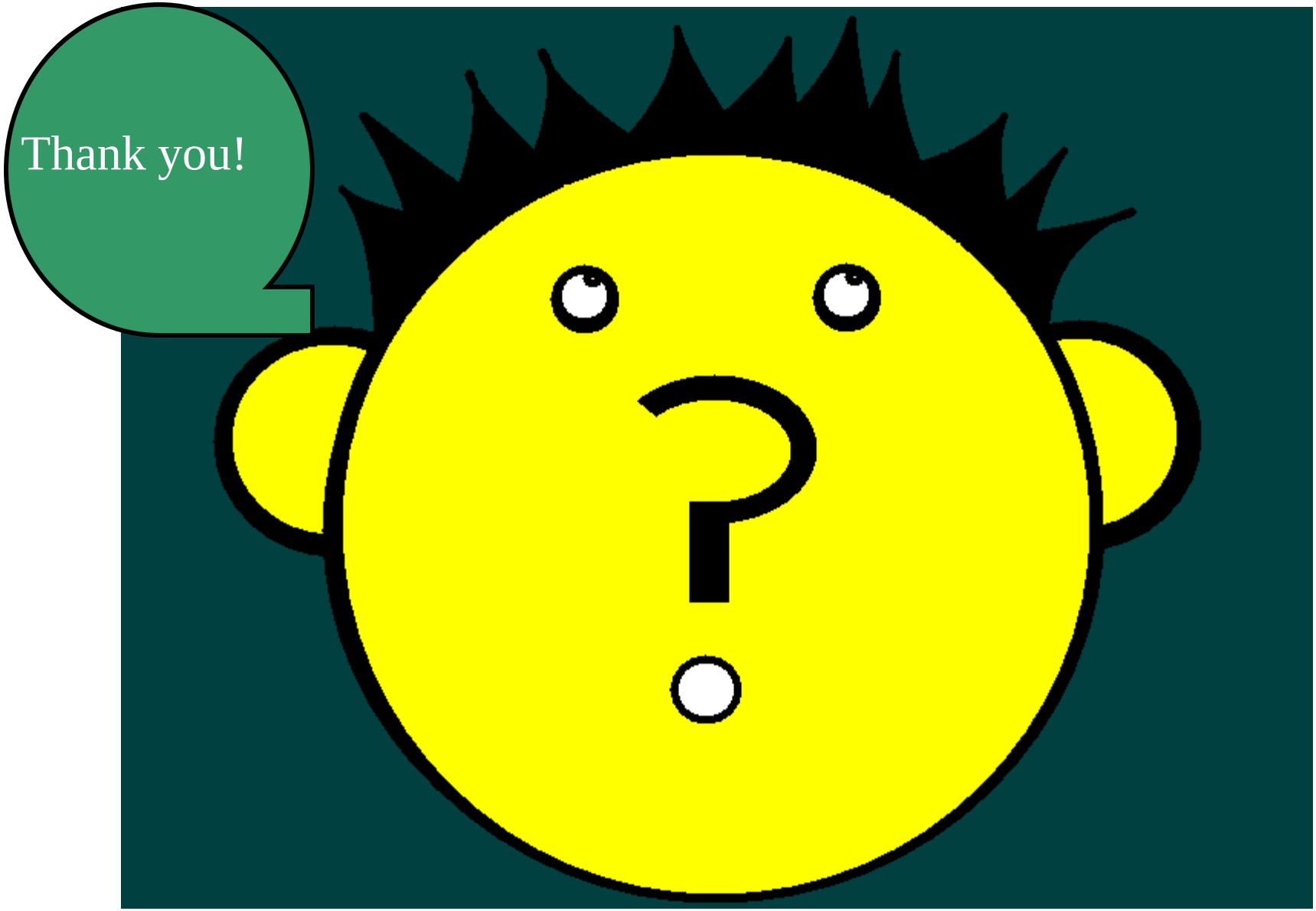
Thank you!